Biological Control of Weeds
Joe Balciunas Research Report
(January 2001 through March 2002)

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The Cape ivy gall fly, *Parafreutreta regalis*.

Executive Summary

This report summarizes the research during the 15 months (January 2001 through March 2002) that was directed by Dr. Joe Balciunas and his South African collaborators. Dr. Balciunas is a Research Entomologist, specializing in biological control of weeds, at the USDA-ARS Western Weeds Quarantine Facility in Albany, California [see Section I for a brief history of this containment facility]. The quarantine, now administratively part of the Exotic & Invasive Weed Research Unit [**EIW**], which has nine principal investigators, or supervisory scientists [**SY's**], at three western locations – three at Albany focusing on developing biological controls for weeds; two in Davis, California researching aquatic weeds; and four SY's in Reno, Nevada studying the ecology of rangeland weeds.

The Cape Ivy Project grew considerably during 2001, and became the primary focus for Dr. Balciunas's research [see Section II]. A significant milestone was reached with the first Cape ivy insects arriving in California, and host range evaluations of these insects beginning in both Albany and Pretoria. In January 2001, Dr. Balciunas, returned from South Africa hand-carrying pupae of the Cape ivy stem moth (*Digitivalva* new species) and galls containing larvae of the Cape ivy gall fly (*Parafreutreta regalis*). Colonies of both were established in our quarantine facility at Albany, and once sufficient numbers were available, host range evaluations began.

The gall fly, *Parafreutreta regalis*, is a fruit fly (family Tephritidae) that appears to specialize on Cape ivy. The female *Parafreutreta*, about the size of a housefly, generally lays eggs inside a node near the growing tip of Cape ivy. The little maggot that hatches within the node causes Cape ivy to grow a spherical structure, about a ½ inch in diameter, within which the maggot completes its life cycle. These galls seem to inhibit further elongation of that stem, although side shoots are usually produced. The weight of the gall causes the stem to droop, and most galls are beneath a "mat"of Cape ivy. We theorize that "galled" Cape ivy plants will be less aggressive in clambering over native trees and shrubs. In Pretoria, the PPRI scientists began parallel studies, and between the two facilities, we have now tested the gall fly on over forty relatives of Cape ivy. So far galls have only been produced on the target – Cape ivy.

Since the impact of galls on Cape ivy plants is subtle, pre-release impact assessments began at the Albany quarantine. The first Impact Trial demonstrated that for small Cape ivy plants, attack by the gall flies result in shorter plants with fewer nodes that have more small leaves, at the expense of full-size leaves.

Digitivalva new species (previously identified as *Acrolepia* new species) was discovered during our surveys and appears to be new to science. It is, however, one of the most widely distributed of Cape ivy natural enemies, and we collected it at nearly all our Cape ivy sites in South Africa. This tiny moth (less than ¼ inch in length) lays eggs within a leaf of Cape ivy. Minute caterpillars hatch out and tunnel within the leaves, leaving distinctive, narrow "mines". Some of the caterpillars bore down through the leaf petiole, and then bore inside the stem of Cape ivy. In the lab, most of the mined leaves, and many of the bored stems die, and sometimes the entire Cape ivy plant is killed. However, since the stem moth has a longer life cycle [approximately three months from egg to adult moth, versus two months for the gall fly], we have completed fewer tests [six]. Also slowing the testing, is the fact that, for unknown reasons, almost 50% of the time the stem moths fail to oviposit on our Cape ivy control plants. These

tests then need to be repeated. Thus far, no plant, other than Cape ivy, appears to be acceptable for oviposition by females of the stem moth.

Our colony of the stem moth nearly died out during summer, and we required an additional shipment from South Africa. Unfortunately, the terrorist attack of Sept 11th resulted in a temporary ban on shipments of live organisms into the USA. However, through his contacts with officials at the American Embassy in Pretoria, Dr. Balciunas was able to arrange for another shipment of moths through diplomatic channels. We now have a strong colony in Albany, and our testing is proceeding at an optimal pace. In fact, we now suspect that this moth actually prefers cooler winter temperatures. This shows great synchronicity with its host, Cape ivy, which also flourishes best through winter and spring.

Now that we have these first two Cape ivy insects in our quarantine, we will begin a lengthy investigation into their host specificity. We must be confident of the safety of any insect we seek to release to control Cape ivy. To speed up this process, our South African cooperators will conduct host-specificity tests of these two insects, in parallel with us in Albany. Several years of laboratory and field evaluations of their host range will be required. Then, if the insect is still deemed safe, we will prepare a request for the release. As regulatory approval for release can easily take an year, it will probably be two to three more years before the first of these insects is released in California.

During the past year, our South African colleagues also extensively tested a third insect, the Cape ivy defoliating moth (*Diota rostrata*). The hairy caterpillars of this moth are voracious feeders, and in South Africa, we frequently encounter patches of Cape ivy where most the leaves are either totally missing or only small tatters remain. Over three dozen species of plants have been tested in South Africa, and the caterpillars of this defoliating moth fed on less than a handful. Unfortunately, this included one of the three California native *Senecio* plants that the South Africans tested. Our South African colleagues plan to confirm the degree of attack on *Senecio flaccidus*, but the introduction of this moth into the USA is now starting to appear doubtful.

Furthermore, we have selected several sites in California where we hope to release our biocontrol agents once they are approved for release. Studies on Cape ivy insect fauna were initiated at two of these sites. We hope a better understanding of the current insect fauna of Cape ivy will allow us to more accurately assess the damage caused by our biocontrol agents once released We completed an year-long field study of the insects attacking Cape ivy at two sites in Monterey County. Every six weeks, we spent several days examining nearly 10 pounds of Cape ivy that we collected from these two sites. All of these samples were nearly devoid of insects or insect damage. This confirms that our California insects are not utilizing this now abundant plant, and that the biocontrol project should proceed.

During this period, Dr. Balciunas also completed the transition of transferring the leadership of the biological control of yellow starthistle project to Dr. Lincoln Smith, who joined our Research Unit in the Fall of 2000. We did, however, complete some long term studies on the host range and biology of the fly *Chaetorellia succinea* [see Section III], an important natural enemy of yellow starthistle, whose larvae severely damage the heads of this weed. The results of this research are being prepared for publication.

January 2001 through March 2002 Research Report

prepared by Joe Balciunas, Stefan Neser, Liamé van der Westhuizen,
Chris Mehelis, and Maxwell Chau

Research presented in this report was performed under the guidance of Dr. Joe Balciunas.

List of Staff and Cooperators

USDA Staff:
Joe Balciunas, Project Leader (Jan. 1, 1996 to present)
Chris Mehelis, Biological Science Technician (April 28, 1998 to present)
Maxwell Chau, Biological Science Technician (March 1, 1999 to present)
Eve Lednicky, Biological Science Technician (July 7, 1999 to present)

PPRI Staff in South Africa:
Stefan Neser, Principal Investigator
Liamé van der Westhuizen, Agricultural Research Technician
Johanna Wing, MS student (Wits University, Johannesburg)

Cooperators:

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Bureau of Land Management – Fort Ord
California Agric. Commissioners & Sealers Assoc.
California Dept. Food & Agriculture (CDFA)
California Exotic Pest Plant Council
California Native Plant Council
California State Parks and Recreation
California Polytechnic State University, San Luis Obispo
East Bay Municipal Utility District (EBMUD)
Golden Gate National Recreation Area
National Park Service – Redwood National Park
Nez Perce Indian Tribe – Bio-Control Center
Oregon Dept. Agriculture (ODA)
Plant Protection Research Institute – Pretoria, SOUTH AFRICA
Rio Pedros Reserve, Carmel, CA
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USDA-ARS, Reno, Nevada
USDA-ARS, Fresno, California
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USDA-Forest Service, Siskiyou National Forest
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Wits University – Johannesburg, South Africa

List of Acronyms and Abbreviations

List of Acronyms

ARS	Agricultural Research Service (an agency of USDA)
APHIS	Animal and Plant Health Inspection Service (an agency of USDA)
CA	California
CalEPPC	California Exotic Pest Plant Council
CDFA	California Department of Food and Agriculture
CINWCC	California Interagency Noxious Weed Coordinating Council
CNPS	California Native Plant Society
CSIRO	Commonwealth Scientific and Industrial Research Organization
EBCL	European Biological Control Laboratory, USDA-ARS
EIW	Exotic & Invasive Weed Research Unit, USDA-ARS, Albany, California
FY	Federal Fiscal year (Oct. 1 to Sept. 30)
ID	Idaho
IPM	Integrated Pest Management
NRA	National Recreation Area
NV	Nevada
OR	Oregon
ODA	Oregon Department of Agriculture
PPRI	Plant Protection Research Institute (an agency of the Agricultural Council of the Republic of South Africa)
TAG	Technical Advisory Group for Biological Control of Weeds
USDA	United States Department of Agriculture
WA	Washington
WRRC	Western Regional Research Center - USDA - Albany, California
YST	Yellow starthistle, *Centaurea solstitialis*

List of Generic Abbreviations

Crd.	*Carduus* thistles
Cnt.	*Centaurea* knapweeds and starthistles
Ch.	*Chaetorellia* flies
Cir.	*Cirsium* thistles
Del.	*Delairea* ivy
Di.	*Digitivalva* moths
Eury.	*Euryops* shrubs
Pa.	*Parafreutreta* flies
Sen.	*Senecio* plants

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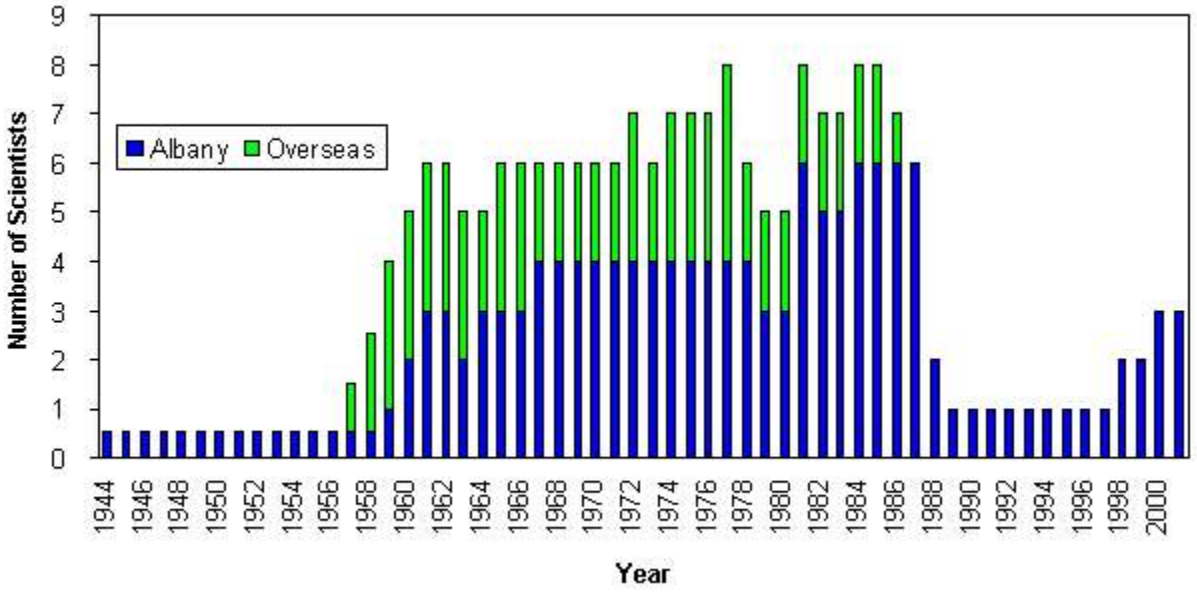
I. The Western Weeds Quarantine Facility

Widespread exotic pests are obvious targets for classical (importative) biological control, which attempts to re-associate an exotic pest with carefully selected and screened natural enemies from its native range. Potential biocontrol agents for weeds undergo extensive host range testing to assure their safety to the environment. In the U.S., prior to release, an array of federal and state agencies review the host range tests and other information about the proposed agent. Ultimately formal approval for release is granted by the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS), as well as by the state(s) where the release will take place. The feasibility of using classical biological control as a tool in managing exotic weeds was first demonstrated during the late 1920's and early 1930's, with the control of prickly pear cactus in Australia with *Cactorum*, a moth introduced from South America. Another Australian project, the control of *Hypericum perforatum*, caught the interest of Professor Harry Smith, Univ. of California - Berkeley, and Jim Holloway, USDA Bureau of Entomology and Plant Quarantine (now part of ARS). This bush - known in Oregon and Washington as "goat weed", and as "Klamath weed" in northern California - now has the "approved" common name of "St. John's wort." This European native is widely established at many temperate locations throughout the world, and had become a severe problem in the western United States, infesting over two million acres in California alone. They set up a cooperative project, and in 1944, imported three species of chrysomelid beetles, which the Australians had earlier imported from Europe for the control of this weed. One of the chrysomelid leaf beetles, *Chrysolina quadrigemina*, established readily and soon provided excellent control of Klamath weed in California, reducing infested acreage to one percent of the former level. Control of this weed in other parts of the Northwest, such as Washington and Oregon, has been less satisfactory, even after the release of several other agents.

The success of the *Hypericum* project led to the construction, in 1963, of USDA's first biological control of weeds laboratory, several miles NW of the Univ. of California's Berkeley Campus, on the University's Gill Tract in Albany. This particular site was chosen because of its proximity to the University's Division of Biological Control, easy access to the University libraries, proximity to an international airport, and its climate. The Mediterranean-type climate allowed the lab to work on weeds occurring throughout the entire U.S., even weeds in such diverse areas as Florida, the arid southwest, the Pacific Northwest, and the Great Plains. Dr. Holloway served as USDA's Director of this facility until his death in 1964. Dr. Lloyd Andres succeeded him and remained in that role until his retirement in 1988. The period from the mid-sixties through the early eighties was a golden era for weed biocontrol in the U.S. (Figure 1). The Albany scientists imported and tested over 70 insect species, of which 54 were released against 24 weed targets throughout the U.S. (Balciunas and Mehelis, 1999). Successful biological control projects during this period were tansy ragwort (*Senecio jacobaea*), puncturevine (*Tribulus terrestris*), alligatorweed (*Alternanthera phylloxeroides*), musk thistle (*Carduus nutans*), and water hyacinth (*Eichhornia crassipes*). Also, during this period, the number of scientists in the United States, working on weed biocontrol increased to 15, of which the four scientists stationed at Albany served as a nucleus, directing many of the domestic projects throughout the U.S., as well as all of the foreign research programs.

Figure 1. Number of USDA-ARS scientists (SY's) working on biological control of weeds at Albany, and assigned overseas, from 1944 through 2001.



In 1986, USDA scientists moved nearby to a new quarantine facility at USDA's Western Regional Research Center. Ironically, in 1987, most of the staff were transferred to a new ARS weed lab in Bozeman, Montana, leaving the new Albany facility nearly vacant. Following Dr. Andres' retirement in 1988, Dr. Charles Turner was left as the sole scientist at the Albany quarantine. Despite several attempts at closing the quarantine facility, Dr. Turner was able to continue biocontrol research there through 1995.

At the end of 1995, after 11 years in Australia conducting research on biological control of *Hydrilla* and *Melaleuca*, Dr. Joe Balciunas transferred to the quarantine from USDA's Australian Biological Control Laboratory. In a "job swap" with Joe, Dr. Charles Turner became the Director of ABCL in January 1996. Tragically, Charley was diagnosed with cancer at the end of his first year in Australia, and he succumbed to the disease after returning to his mother's home in Indianapolis, Indiana, on April 15, 1997.

Although Dr. Balciunas was able to obtain new, outside resources for research on new targets, "base funds" from USDA for the quarantine continued to be problematic. Insufficient base funding at the end of our federal fiscal year in Sept. 1997 led to the loss of our only permanent support staffperson, Kathy Chan. This resulted in postponement of the host range testing in quarantine containment, and the focusing on field evaluations of *Chaetorellia* flies. Early in 1998, USDA permanently increased the base funds for the yellow starthistle project in Albany by $50,000 annually. Thus, at the end of April 1998, we were able to advertise and hire a replacement, permanent technical assistant, Mr. Chris Mehelis, and we quickly resumed quarantine evaluations of potential agents for YST and Scotch thistle. Joe was able to add another full-time assistant, Maxwell Chau, in March of 1999, and a ½ time greenhouse assistant, Eve Lednicky, in July 1999.

There were additional positive changes for our Research Unit. In mid 1998, the one-scientist project at Albany was administratively combined with the two scientists in Davis, California studying aquatic weeds and the three scientists in Reno, Nevada who study range management, into the new Exotic & Invasive Weed Research Unit. At the beginning of July 1998, Dr. Ray Carruthers, an entomologist, who for the previous four years had served as the ARS National Program Leader for Biological Control, was added to our unit, and and continues to serve as Research Leader. Ray is based in Albany, and in addition to his administrative tasks, conducts research on biological control of saltcedar (*Tamarix* spp.) and giant reed grass (*Arundo donax*).

During the past three years, 1999-2001, the Exotic and Invasive Weed Research Unit has continued to expand. At the end of 2000, the Research Leader's assistant, John Herr, took over the Quarantine Officer role. In October 2000, a third Research Scientist (SY) was added to the biocontrol of weeds group in Albany. Dr. Lincoln (Link) Smith, a Research Entomologist, formerly with the USDA-ARS biological control of weeds group in Sidney, Montana, accepted the position at Albany to work on biological control of yellow starthistle (YST) and Russian thistle (also known as tumbleweed). Joe has now focused his research on the expanding Cape ivy project, and has turned the reins to the YST project over to Link.

At the end of 2001, Dr. Carla d'Antonio, an ecologist from University of Calfornia - Berkeley, joined our Research Unit, and in mid-2002, will join the other three research scientists at our Reno location.

II. Cape ivy (*Delairea odorata,* prev. *Senecio mikanioides*) research

A. Introduction

Cape ivy (also known as German ivy), a native of South Africa, has recently become one of the most pervasive and alarming non-native plants to invade the coastal areas of the western United States. Botanically, this plant is a member of the sunflower family (Asteraceae), and, in the U.S., is still frequently referred to by its old name, *Senecio mikanioides.* However, its accepted scientific name in most other countries is *Delairea odorata.* A recent survey (Robison *et al.* 2000) reports Cape ivy infestations from San Diego to southern coastal Oregon. Cape ivy is spreading in riparian forests, coastal scrubland, grassland, Monterey pine forest, coastal bluff communities, and seasonal wetlands. Though the species prefers moist, shady environments along the coast, there are increasing reports of infestations from inland riparian locations. This vine has the potential to cause serious environmental problems by overgrowing riparian and coastal vegetation, including endangered plant species, and is potentially poisonous to aquatic organisms.

Cape ivy has become the highest-ranked invasive species problem in the Golden Gate National Recreation Area (NRA). Golden Gate NRA spent a $600,000 grant over three years for Cape ivy control efforts. California State parks on the coast are heavily impacted as well. These include Big Basin, Hearst San Simeon, Mt. Tamalpais, Van Damme and Jughandle. U.S. Forest Service lands along the Big Sur coast are heavily impacted, as are lands of many other agencies on the coast.

Cape ivy was introduced into the Big Island of Hawaii around 1909 and has become a serious weed in a variety of upland habitats there, between 200 and 3000 meters elevation. (Jacobi and Warshauer 1992). Two reports (Haselwood and Motter 1983, Jacobi and Warshauer 1992) state that in the Hawaiian Islands this vine is restricted to the Big Island. However, Wagner *et al.* (1990) state that it is also sparingly naturalized on Maui.

B. Overview of collaborative research in South Africa (1996 to 2002)

On his first trip to South Africa during 1996, Dr. Balciunas did a thorough study of South African Cape ivy herbarium records. These records were used to locate Cape ivy sites for future surveys and to develop a distribution map of Cape ivy in South Africa (Balciunas *et al.*, 2001). Since 1997, CalEPPC and the California Native Plant Society (CNPS), have raised funds to assist our USDA-ARS project on the biological control of Cape ivy. Together CalEPPC and CNPS have been successful in raising $30,000-65,000 annually. We have used these contributions to contract research in South Africa, the native home of Cape ivy, and we have been fortunate enough to obtain the services of Dr. Stefan Neser, a world-renown biological control specialist, as well as several talented younger, South African scientists for this project.

Each year, Dr. Balciunas has spent 4-5 weeks with our South African cooperators, reviewing their results, assisting in the current research, and jointly planning the research for the following year. Dr. Balciunas visited South Africa again in Aug. of 1998, during the first year of collaborative research there (March 1998 to March 99). He joined our South African cooperators, and participated in a 3000 km survey led by Beth Grobbelaar and Stefan Neser, that

visited most of the Cape ivy sites in the country, and collected the natural enemies that attacked it. Over 230 species of plant-injuring insects were collected at these sites (Grobbelaar *et al.*, in press).

Six of the most promising of insects were selected for further research. These included: *Diota rostrata* (Arctiidae) - a defoliating caterpillar; *Digitivalva* new species – a stem boring/leaf mining moth caterpillar; *Parafreutreta regalis* (Tephritidae) - a stem galling fly; an unidentified leaf mining Agromyzid fly; and two species of Galerucine leaf beetles (Chrysomelidae) - which feed on leaves as adults or larvae. During the second year (April 1999 to March 2000), our South African team tried to collect these six insects on relatives of Cape ivy growing at these sites. More than a dozen close relatives of Cape ivy were repeatedly examined, but only one of the six insects, the arctiid moth - *Diota rostrata*, was collected on anything other than Cape ivy, and so it appears that at least five insects are very host-specific to Cape ivy.

The focus of the third and fourth years of research (April 2000 to March 2002) was to further scrutinize the host range of these promising insects. This phase of research was led by South African weed biocontrol specialist, Dr. Stefan Neser, and his assistant Liamé van der Westhuizen. They were able to establish laboratory colonies of two Cape ivy insects: *Digitivalva* new sp., and *Parafreutreta regalis*. In addition, Joanna Wing, a USDA-sponsored graduate student at Wits University in Johannesburg, studied the biology of the arctiid moth, *Diota rostrata*. Neser and van der Westhuizen also compiled valuable information on the biology and life history of these three insects, and developed rearing techniques.

In December 2000, Dr. Balciunas visited South Africa for the fourth time. While in South Africa, Dr. Balciunas was briefed on research by South African scientists and learned the biology of the insects selected from previous years insect surveys as potential biological control agents. He brought back two of these potential biocontrol agents for evaluation in our quarantine laboratory. These two insects: the gall forming fly - *Parafreutreta regalis*, and *Digitivalva* new sp. were discovered on previous trips to South Africa, damaging and causing deleterious effects to Cape ivy in South Africa.

C. Field research on Cape ivy plants in California

1. Seed germination

Tests of the seeds produced by Cape ivy in California have generally shown that they are not viable – they generally will not germinate and produce a seedling. For instance, Carla Bossard (2000) reports that none of the thousands of seeds, from 26 California populations, examined by her and her students, were viable. Young, Balciunas, and Clements [Proceedings, 2000 CalEPPC Symposium] likewise report that although Cape ivy seeds from South Africa and Hawaii germinated readily, those from California failed to germinate.

Nevertheless, new Cape ivy populations keep appearing at locations where it seems highly implausible that they generated from fragments of Cape ivy. Thus many weed warriors, e.g. Jake Sigg (1993) have insisted that Cape ivy in California must at least occasionally produce viable seed.

To help resolve this question, during last year's CalEPPC Symposium in Concord, I offered a one hundred dollar reward to the first person who could provide me with viable Cape

ivy seeds from California. This reward has been claimed. In February 2001, Matthew Simone, a volunteer, was inspecting a recently cleared infestation of Cape ivy at Ft. Cronkite in the Marin Headlands for resprouts and overlooked plants. He noticed some tiny Cape ivy plants that appeared to be seedlings. He brought these to the attention of National Park Service Cape ivy team leader, Ellen Hamingson. She was aware of my reward offer, and phoned me about Matthew's discovery. A few days later, on February 15th, I met Ellen at Ft. Cronkite, and, accompanied by Mona Robison, inspected some of the seedlings, both in pots and at the field site. By this time, the plants were several inches high, and had a half dozen true leaves. It was, therefore, difficult at that time, to confirm that these small plants had grown from seeds, rather than plant fragments.

However, less than 50 yards from the site, we found some Cape ivy that had just finished flowering. We collected some of the most promising heads – with the receptacles mostly brown and senescent, but still closed and clasping the white"powder puffs" of pappus, the silky hairs to which the seeds, if any, would be attached. Back at my laboratory in Albany, my assistant Eve Lednicky, split the heads from this sample. As usual, the heads contained mostly shriveled seeds, but this time, there was an occasional large, plump seed. We planted several dozen of these promising, plump seeds in commercial potting mix. Within two weeks 11 seedlings had sprouted. The photo (Figure 2) shows one of these seedlings after about month. It has put out its first true leaves, but beneath these, the dicotyledon leaves are still apparent. It unquestionably sprouted from a seed.

Figure 2. One month old Cape ivy seedling, sprouted from seed collected in Marin County California by Dr. Joe Balciunas

Mona Robison (2001) later tested the viability of Cape ivy seeds collected from 50 sites in California and Oregon, and confirmed that viable seeds were present at all sites. In California, the flowering period of Cape ivy varies from site to site, and from season to season. However, the first flowers can appear as early as October, the peak flowering is usually in December and January, and the last flowers linger into March.

2. California Cape ivy insect surveys

We initiated a project in May of 2001 to determine which herbivorous California insects have accepted the now abundant Cape ivy as a host. We surveyed Cape ivy at two sites near Monterey approximately every six weeks, and once, a site at Pt. Isabella in Contra Costa County. Each collection consisted of two samples – one cut from a horizontal portion of a mat, the other removed from a portion of the infestation that formed a near-vertical curtain covering surrounding trees and shrubs. The horizontal samples were approximately 0.5 by 0.5 meter, while the vertical sample was 0.3 by 2.0 meters. The thickness of the sample varied with the season but was generally, 0.1 to 0.2 meters thick. A machete and pruning shears were used to cut the samples from the mat, and the samples were placed into large paper sacks, closed with large clips, then transported back to our lab in large, insulated coolers.

Processing the samples back at our laboratory was quite tedious, and each sample required nearly a day's worth of time by two or three of my assistants. We first divided the plants into leaves, stems, dead material, and floral parts (if present), then examined each of these plant portions for insects or their damage. Insects found were recorded, and if adults, were either pinned or preserved in 70% EtOH, except when many had already been collected (i.e. aphids, collembola, psocopterans, etc.). We attempted to rear all immature insects to adults, but usually with little success. Once the examinations of the samples were complete, we placed the samples (separated into leaves, stems, and dead material) into emergence boxes, which are sealed cardboard boxes (36.5 cm^3) mounted with a protruding clear plastic shell vial. The vial provides a small source of light in the box which attracts emerging adults. We monitored these boxes for newly emerging insects for one to two months.

Table 1 shows the results of these surveys. Generally, we found very few herbivorous insects in these samples, and the insects found appear to be generalists, such as aphids and book lice.

Table 1. Results of the California Cape ivy surveys.

Date	May 22				Jun 12		Jul 10				Aug 21				Oct 15				Jan 15 (2002)						Apr 16	
Site	MW backyard vertical	MW backyard horizontal	RP vertical	RP horizontal	Point Isabella vertical	Point Isabella horizontal	MW backyard vertical	MW backyard horizontal	RP vertical	RP horizontal	MW backyard vertical	MW backyard horizontal	RP vertical	RP horizontal	MW backyard vertical	MW backyard horizontal	RP vertical	RP horizontal	MW backyard vertical	MW backyard horizontal	MW backyard flowers	RP vertical	RP horizontal	RP flowers	RP vertical	RP horizontal
Plant portions																										
leaves (g)	172	126	359	78	87	117	422	113	302	276	223	48	164	18	425	119	141	105	771	208	115	352	142	82	644	296
stems (g)	300	189	1029	433	640	137	552	297	1765	594	394	184	445	455	649	620	740	924	1371	421	139	952	915	97	1099	844
flower buds (g)																			5	1	16	0	0	0		
flowers (g)																			16	29	132	0	0	37		
dead flowers (g)																			2	4	0	0	325	62		
dead material (g)	6	2	70	44	73	13	64	79	1032	273	65	3	144	921	110	105	233	312	126	29	16	74	1	7	64	246
Insects and other arthropods																										
Acarina		X	X	X								X	X	X			X	X	X		X		X		X	
Coleoptera:																										
undetermined family																			X	X			X			
Curculionidae																				X	X					
Staphylinidae																			X							
Collembola	X	X	X	X								X		X			X									
Dipteran																			X				X			
Gastropoda (snails)												X		X				X	X							
Psocoptera	X	X	X		X	X	X	X	X		X				X			X	X	X					X	
Homoptera:																										
Aphididae	X	X	X				X		X	X	X				X				X	X	X	X	X	X	X	X
Cicadellidae	X	X	X	X		X	X	X	X	X	X		X	X		X	X	X							X	X
Cercopidae	X		X	X																					X	X
Psyllidae						X									X				X	X		X				
Hemiptera: Miridae		X	X															X	X						X	X
Hymenoptera																			X							
Lepidoptera:																										
Geometridae							X		X										X						X	
blister moths	X	X	X				X								X											
undetermined family	X						X		X	X				X		X		X		X		X				X

D. Laboratory research in the United States and South Africa on potential biological control agents for Cape ivy

During the past 15 months, the research at our Albany facility, as well as in Pretoria, has concentrated on evaluating the safety and potential of some of the insects discovered during the first two years of surveys in South Africa.

Safety is the primary concern for those involved in releasing herbivorous insects from overseas. Everyone wishes to feel confident that the insects are narrowly host-specific – that once they are released and established, they will not cause significant damage to native plants, crops, or desirable ornamental plants. The degree of host-specificity of candidate insects is usually determined by exposing the candidate insects, usually in cages in the laboratory, to an array of potential host plants, and then noting which of these, if any, are also suitable as hosts. Traditionally, these laboratory host range evaluations are comprised of "no-choice tests" [sometimes called "starvation tests] in which the known host (in this case Cape ivy) is not present in the cage, and of "choice tests" where the target host is present.

1. Host range tests of *Parafreutreta regalis,* the Cape ivy gall fly

The gall fly, *Parafreutreta regalis*, is a fruit fly (family Tephritidae) that appears to specialize on Cape ivy. The female *Parafreutreta,* about the size of a housefly, generally lays eggs inside the growing tip of Cape ivy. The little maggot that hatches within the tip causes Cape ivy to grow a spherical structure, about a ½ inch in diameter, within which the maggot completes its life cycle. These galls seem to inhibit further elongation of that stem, although side shoots are usually produced.

a. USDA research in Albany, CA

Parafreutreta regalis adults are short-lived, averaging around 7 days, and the test plants that we wish to use are limited in number, and are usually seasonal. Using traditional approaches, would mean that completing the planned array of tests would require many years. Accordingly, Dr. Joe Balciunas, in consultation with our South African cooperator, Stefan Neser, designed a rather unique protocols for host range tests that allow us to maximize the data from whatever flies and test plants are available. Essentially, these tests (that we call "**no-choice/host added**") are a multi-plant no-choice test, to which, after three days, a Cape ivy plant is added. The procedures we used in Albany [our collaborators in Pretoria used nearly identical protocols] are as follows: a metal screen cage (122 x 91½ x 91½ cm) was set up in our quarantine laboratory greenhouse with four different plant species, one in each corner. A source of sugar water (50% Mt. Dew [a soda produced by Coca Cola Co.] and 50% water in a shell vial with a wick) was placed in the center of the cage. We released four female-male pairs of flies into the cage. The flies used were young, always within two days of emergence as adults. We believe that *Pa. regalis* females are ovipositional soon after emergence. In 2002, we began experiments to confirm this. In preliminary observations, we have noted, in a few instances, oviposition on Cape ivy by *Pa. regalis* as early as 24 hours after emergence, although usually, oviposition is

seen with 48 hours of emergence. Three days later, we placed a Cape ivy plant into the center of the cage. Four days after that (seven days after the start of the test), we ended the test. Flies were recovered and preserved as voucher specimens. All plants were held and observed daily, for signs of gall formation. If no galls had formed after 60 days we dissected the stems looking for signs of *Parafreutreta* damage, then disposed of the plants. Table 2 summarizes the "no-choice/host added tests", while details of the tests can be found in Appendix B.

Table 2. *Parafreutreta regalis* no-choice/host added test results for Albany.

Test Plant	N	Mean no. galls per plant	Total adult emergence
Delairea odorata	14	5.6	110+ females, 96+ males
Adenocaulon bicolor	4	0	0
Erechtites glomerata	3	0	0
Euryops pectinatus	3	0	0
Euryops subcarnosum	5	0	0
Hedera canariensis	3	0	0
Luina hypoleuca	2	0	0
Senecio blochmaniae	4	0	0
Senecio bolanderi	3	0	0
Senecio breweri	4	0	0
Senecio confusus	4	0	0
Senecio flaccidus	4	0	0
Senecio ganderi	2	0	0
Senecio hybridus	3	0	0
Senecio jacobaea	2	0	0
Senecio macounii	4	0	0
Senecio triangularis	3	0	0

We were able to conduct 24 no-choice/host added tests with *Pa. regalis* from Jan. 2001 through March 2002. Fourteen of those tests showed a positive control – galls developed on the Cape ivy, thus confirming that indeed the flies used in the test were ovipositional. None of the other 16 species of test plants exposed in those fourteen tests, showed any sign of *Pa. regalis* oviposition. We did not find galls or any sign of *Pa. regalis* damage on any plants other than Cape ivy. So far it seems that the host range of *Pa. regalis* is restricted to Cape ivy. We plan to conduct more no-choice/host added tests on more related plant species to confirm *Pa. regalis*' apparent restricted host range.

We are also interested in determining whether or not there is an ovipositional preference by *Pa. regalis* on Cape ivy from different regions (California, Hawaii, and South Africa). In November we started choice tests on Cape ivy using similar protocols as our no-choice/host added tests. Instead of using four non-target test plants, we started with four Cape, but did not add an additional Cape ivy once the test was started.

We have completed four tests so far: two on Californian and South African Cape ivy, and two on Californian and Hawaiian Cape ivy. South African Cape ivy seems morphologically distinct from Cape ivy from other locales (it can be easily told apart by appearance). Table 3 shows the results of these choice tests.

Table 3. *Parafreutreta regalis* choice tests on Cape ivy from different regions.

Test No.	Cape ivy origin	No. of galls			Adult emergence from galls
		Plant 1	Plant 2	Total	
19-01	California	10	9	19	40 females, 22 males
	South Africa	1	0	1	1 female
20-01	California	7	3	10	9 females, 6 males
	South Africa	6	5	11	2 female, 1 male
21-01	California	11	5	16	8 females, 14 males
	Hawaii	8	2	10	15 females, 12 males
22-01	California	5	5	10	4 females, 9 males
	Hawaii	1	5	6	1 female, 1 male

So far, the results of these tests do not seem to indicate a preference for Cape ivy from a particular region. We will continue these tests and, hopefully, will be able to confirm that no significant difference exists.

b. Host range tests by PPRI in South Africa

The host range tests of *Pa. regalis* conducted in Pretoria were also no-choice/host added tests, and were very similar to those conducted in Albany. Three or four test plants of roughly similar size were placed in a cage (560mm x 560mm x 600mm) with four pairs of newly emerged flies for 3 days. Flies were provided with a honey and yeast solution. On day four, the control, a Cape ivy plant of similar size, was added to the cage. After another 3 days of exposure, the flies were removed and plants were left in the cage and gall development monitored. These trials were replicated 4 times for each test species.

Table 4. Plants exposed to egg-laying females of *Parafreutreta regalis* in no-choice/host added tests in Pretoria, South Africa. All tests were replicated 4 times.

Genus and species	Common name	Family if not Asteraceae	Asteraceae Tribe	Fleshiness of leaves
		Same sub-tribe as *Delairea odorata*		
Delairea odorata	Cape ivy		Senecioneae	++
Euryops pectinatus	grey-leaved euryops		Senecioneae	0
E. chrysanthemoides	golden daisy		Senecioneae	0
Mikaniopsis cissampelina			Senecioneae	++
Senecio angulatus			Senecioneae	++
S. bolanderi (USA)			Senecioneae	0
S. brachypodus			Senecioneae	++
S. deltoideus			Senecioneae	0
S. flaccidus (USA)			Senecioneae	+
S. helminthioides			Senecioneae	++
S. oxyodontus			Senecioneae	++
S. oxyriifolius			Senecioneae	++
S. pleistocephalus			Senecioneae	++
S. tamoides	canary creeper		Senecioneae	++
Senecio sp. (unidentified)			Senecioneae	++
		Other Senecioneae		
Cineraria cv "butterfly"			Senecioneae	0
C. deltoidea			Senecioneae	0
C. lobata			Senecioneae	+
		Commercially important Asteraceae		
Lactuca sativa	lettuce		Lactuceae	+
		Commercially important non-Asteraceae		
Brassica oleracea	cabbage	Brassicaceae		+
Raphanus sativus	radish	Brassicaceae		0
Beta vulgaris var. *cicla*	leaf spinach/Swiss chard	Chenopodiaceae		+
		Other Asteraceae		
Arcoteca calendula			Arctoteae	+
Coreopsis sp. cv.	tickseed		Heliantheae	0
Dahlia pinnata cv	garden dahlia		Heliantheae	0
Rudbeckia sp. cv.	coneflower		Helenieae	0
Tagetes minuta	tall khaki weed		Helenieae	0
Zinnia elegans cv.	zinnia		Helenieae	0
		Widespread weeds (mainly Asteraceae)		
Ageratina adenophora	crofton weed/ Mexican devil		Eupatorieae	0
Ageratina riparia	mistflower		Eupatorieae	0
Galinsoga parviflora	small flowered quick weed		Helenieae	0
Campuloclinium macrocephalum	pompom weed		Eupatorieae	0

In Pretoria, *Parafreutreta regalis* flies were tested on 33 plant species, including several asteraceous species closely related or similar to *Delairea odorata.* Galls were only produced on Cape ivy (Table 4), usually about 10-20 per plant, but the number ranged from 1-45. Although galls developed in growing, young tissue of shoot tips of runners and of side shoots in axils of leaves, most galls were found at the base of shoots at ground level.

During the period of 2000 and 2001 a number of gall measurement and other related data were recorded. Data recorded included gall length, width, and volume (ellipsoid volume = $4/3*pi(\text{length}/2)(\text{width}/2)^2$) as well as the total number of windows, number of males, females and the total number of flies per gall. Using Genstat 5, a statistical analysis was done in order to test for any significant correlation between the variables (Table 5).

Table 5. Correlation between different variables for *Parafreutreta regalis* gall formation.

Volume (ellipsoid)	**1**	1.000						
No. windows	**2**	0.313	1.000					
Length	**3**	0.808***	0.224	1.000				
Width	**4**	0.927***	0.235	0.606*	1.000			
Total no. flies	**5**	0.623*	0.486	0.553*	0.513*	1.000		
Male	**6**	0.480	0.398	0.479	0.383	0.734**	1.000	
Female	**7**	0.505*	0.375	0.405	0.426	0.839***	0.246	1.000
n= 91		**Volume (ellipsoid)**	**No. windows**	**Length**	**Width**	**Total no flies**	**Male**	**Female**

***	Very strong correlation	Tabled critical	r = 0.2061 (p< 0.05)
**	Fairly strong correlation		r = 0.2687 (p< 0.01)
*	Moderate correlation		r = 0.3393 (p<0.001)

There was only a moderate correlation (0.623) between calculated gall volume and number of flies; taking into account that the few strong and fairly strong correlations that were found were to be expected, but do not seem to be of particular importance, no further measurements and counts are planned.

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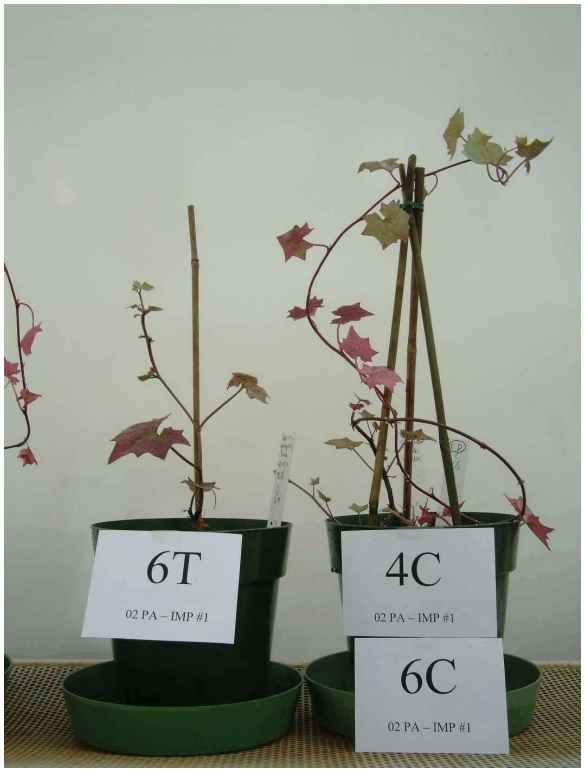
2. Impact assessment of probable damage by *Parafreutreta regalis*

Although *Pa. regalis* readily oviposit and develop galls on Cape ivy, we are concerned that these galls may not significantly impact Cape ivy plants. Even a "safe" biological control agent that is completely restricted to its target weed, may cause unforseen changes to the ecosystem, especially if the populations of the agent build up to high levels, without a corresponding decrease to the target weed. This has already been documented for another tephritid gall fly that was released to control spotted knapweed, *Centaurea maculosa* (Pearson et al. 2000). These researchers found that the extremely abundant galls on knapweed were changing the behavior and populations of deer mice, that had learned to feed on the abundant overwintering larvae of in the galls. Thus, Dr. Balciunas would not request release of *Pa. regalis*, even though it would not damage any other plant than Cape ivy, unless he felt that the damage to Cape ivy might reduce the invasiveness of this vine.

Accordingly, Dr. Balciunas designed and performed a trial to determine if *Pa. regalis* galls reduce the fitness of Cape ivy plants. On Christmas eve 2001, he initiated impact assessment tests to determine if *Parafreutreta* galls reduce the biomass or height of Cape ivy plants. He selected five pairs of small Cape ivy plants that were similar to each other, and placed them in separate double-sleeved, plexiglass cages. In three of the cages, he maintained 10 pairs of *Parafreutreta*, adding flies when necessary, and each of the remaining two cages [two Cape ivy plants] as controls. Aphids quickly became a problem, and all 10 plants needed to be examined daily, and the aphids crushed. Galls formed on all six of the exposed plants, and these plants were obviously more stunted and scraggly (Figure 3).

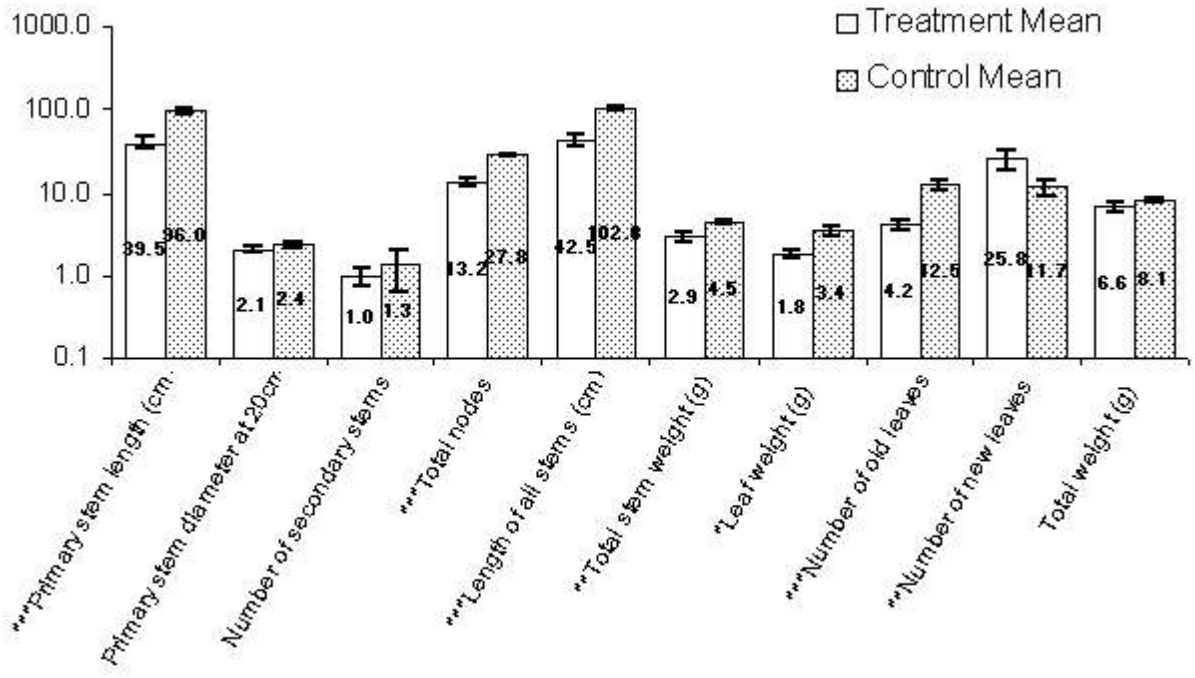
Figure 3. *Parafreutreta* impact assessment test 6: test Cape ivy exposed to *Parafreutreta regalis* and control.



After seven weeks, the experiment was ended, and we weighed and measured the plants, and statistically confirmed this difference between plants with galls, and those without. Our analyses confirmed that the galled Cape ivy plants were statistically shorter, with fewer nodes and smaller leaves (Figure 4).

We have initiated a second impact trial to assess if smaller numbers of flies (2 pairs) can significantly alter the growth of larger Cape ivy plants.

Figure 4. Histogram depicting the differences between six Cape ivy plants exposed to 10 pairs of *Parafreutreta regalis* flies and four similar control plants without flies. Error bars indicate the standard error.



* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Students' T-test.

3. The Cape ivy stem boring/leaf-mining moth, *Digitivalva* new species

Digitivalva new species (initially identified as *Acrolepia* new species) was discovered during our surveys in South Africa, and appears to be new to science. It is, however, one of the most widely distributed of Cape ivy natural enemies, and it has been collected at nearly all our Cape ivy sites in South Africa. This small moth (less than ¼ inch in length) lays eggs within a leaf of Cape ivy. Tiny caterpillars hatch out and tunnel within the leaves, leaving distinctive, narrow "mines". Some of the caterpillars bore down through the leaf petiole, and then bore inside the stem of Cape ivy. In the lab, most of the mined leaves, and many of the bored stems die, and sometimes the entire Cape ivy plant is killed.

a. USDA *Digitivalva* research in Albany

We conducted three different kinds of host range tests (three choice tests, five no-choice tests, and ten no-choice/host added tests) with *Digitivalva* new sp. during 2001 and the first quarter of 2002. For all tests, the adult moths were provided a nutrient source (50% Mt. Dew, 50% water) in a shell vial with a wick. We began our host ranges evaluations of this moth using no-choice and choice tests. Because this moth can be very hard to recover once released into a

cage, we ran both kinds of tests in wooden "double sleeve cages" (size: 72.9 x 41.9 x 48.9 cm) in the quarantine laboratory greenhouse. These wooden cages are box-like, painted white, and the moths were easier to find in these cages. The protocols for each kind of test were as follows. For no-choice tests: the first part of the test (the test plant portion), two non-target test plants of similar condition were placed at opposite sides of the sleeve cage. Three female and three male moths (six total) that had emerged the previous day were added to the sleeve cage. After four days, all moths were recovered, the test plants were removed, and replaced with two Cape ivy plants, each of similar size and condition. The recovered moths used in the first part of the test were then released back into the cage. After three more days (seven total) the test was ended. All moths were recovered and saved as voucher specimens.

The choice tests were similar: two or three plants (one Cape ivy control, and one or two non-target test plants) were put in a sleeve cage. When two test plants were used, they were placed on either side of the Cape ivy control. Three female and three male *Digitivalva* (less than one day old) were added to the sleeve cage. The test was ended after seven days, and the moths recovered and saved as voucher specimens.

The plants used in no-choice or choice tests were then closely monitored for two months for the distinctive signs of *Digitivalva* infestation (leaves with mines, and/or piles of frass coming out of the small holes or cracks in the stems). We recorded the number of adults, if any, that emerged from each plant. Two months after a test had ended, we dissected the stems of the test plants to verify lack of oviposition, looking for dead larvae, or signs of larval damage. The results of these no-choice and choice tests are summarized in Table 6 below, and more details can be found in Appendix C.

Table 6. *Digitivalva* new species no-choice and choice test results for Albany.

Test No.	Type of test	Non-target test plants	No. of *Digitivalva* alive at end of test plant portion of no-choice tests	*Digitivalva* emergence
DI-1-2001	Choice	*Sen. bolanderi, Sen. macounii*	NA	from Cape ivy
DI-2-2001	Choice	*Sen. triangularis*	NA	No emergence
DI-3-2001	Choice	*Sen. bolanderi*	NA	No emergence
DI-4-2001	No-choice	*Sen. triangularis*	4	No emergence
DI-5-2001	No-choice	*Sen. flaccidus*	5	No emergence
DI-6-2001	No-choice	*Sen. blochmaniae*	4	from Cape ivy
DI-7-2001	No-choice	*Petasites frigidus*	4	No emergence
DI-9-2001	No-choice	*Sen. macounii*	5	No emergence

No *Digitivalva* moths emerged form any of the nine test plants used in these three choice and five no-choice tests. Unfortunately, only two of the Cape ivy controls used in these eight

tests produced Digitivalva. We noted a similar reluctance to oviposit on every Cape ivy plant in our colony propagation efforts. For our colonies, we used three to six Cape ivy plants in a cage. Despite continual exposure to many *Digitivalva* for month or more, only a few of the Cape ivy plants in any cage would produce adult *Digitivalva*.

In order to speed up the testing of *Digitivalva*, we began using no-choice/host added tests, similar to the tests used for *Pa. regalis* Four different non-target test plants were set up (one in each corner) in a metal screen cage (122 x 91½ x 91½cm) with a nutrition source (50% Mt. Dew, 50% water in a shell vial with a wick). We then released four pairs of newly emerged (1-2 days old) female and male *Digitivalva* into the cage. Three days later, we added a *Delairea* plant into the center of the cage. Seven to ten days later (depending on the number of moths still alive after seven days), the test was ended by removing all remaining live moths, and recovering the dead moths. Plants were watered as necessary and held for observation.

We checked the Cape ivy and non-target plants daily for signs of *Digitivalva* infestations. When it was apparent that plants were infested, we isolated these plants and collected pupae and adults that emerged from these plants. For plants with no apparent infestation, after sixty days, we dissected the plants to look again for signs of infestation and dead larvae before discarding them. The results of these no-choice/host added tests are shown in Table 7.

Table 7. Successful no-choice/host added tests of *Digitivalva* in Albany.

Test Plant	**N**	***Digitivalva* emergence**
Delairea odorata	6	32♀ & 21♂ *
Euryops pectinatus	3	0
Euryops subcarnosum	3	0
Hedera helix	2	0
Luina hypoleuca	1	0
Senecio bolanderi	2	0
Senecio breweri	2	0
Senecio confusus	3	0
Senecio ganderi	1	0
Senecio hybridus	2	0
Senecio jacobaea	1	0
Senecio macounii	2	0
Senecio triangularis	1	0
Senecio vulgaris	1	0

* from two (of the six) Cape ivy controls.

Out of the 10 no-choice/host added tests we have conducted thus far, we have had *Digitivalva* moths emerge from Cape ivy, and no development on any of the non-target test plants. We plan to continue evaluating this moth using our no-choice/host added test protocols.

In summary, of the 18 host range tests of *Digitivalva* we conducted in Albany, only one no-choice and one choice test showed a positive control - *Digitivalva* new sp. had oviposited on Cape ivy. Six of ten no-choice/host added tests showed positive controls. None of the non-target test plants in any test showed evidence of larval damage or adult emergence.

b. PPRI research on *Digitivalva* in South Africa

Reliable results using the no-choice/host added protocols were not obtained, since oviposition did not take place on the Cape ivy controls, nor on any of the test plants. Test plants that were exposed included: *Senecio angulatus, S. tamoides, S. helminthioides, S. oxyodontus, Cineraria lobata, Mikaniopsis cissampelina* and *Mikania capensis.* Likewise, no indication of egg-laying or development on plants other than *D. odorata* was found in opportunistic observations.

4. *Diota rostrata* (Lepidoptera: Arctiidae) moth tests in South Africa

During the past year, our South African colleagues also extensively tested a third insect, the Cape ivy defoliating moth, *Diota rostrata* (Figure 5). The hairy caterpillars of this moth are voracious feeders, and in South Africa, we frequently encounter patches of Cape ivy where most the leaves are either totally missing or only small tatters remain.

Figure 5. *Diota rostrata* larvae (left) and adult (right).

Almost all developmental stages of *Diota rostrata* are temperature dependent, with higher temperatures resulting in shorter developmental stages. The pre-oviposition period lasts for 2-3 days, after which the female deposits most of her eggs on the underside of leaves, but leaf petioles and plant stems can also be selected. Under laboratory conditions, females will deposit their eggs on the sides of plastic containers. Clusters of eggs can be found with numbers ranging

from 1-56 per group. The eggs are small, round, shiny and yellow in color, and will hatch within 7-15 days. Five larval instars can be distinguished and the larval stage ranges between 21-35 days, and the pupal stage between 9-25 days. Adult longevity is normally 14 days.

Larvae of *Diota rostrata* were collected in the Kirstenbosch Botanical Gardens (Cape Town, South Africa) on *Senecio angulatus* and *S. tamoides*, in June 2001. Specimens were also reared from *S. oxyodontus* there. All the larvae collected were brought back to the laboratory in Pretoria, where their identity was verified, and a new culture of *Diota* established ("Kirstenbosch culture"). Even though Miss J. Wing had already done some host specificity testing with neonate larvae on cut leaves, it was considered to be of great importance to establish whether the Kirstenbosch *Diota* would have the same host preference as their Johannesburg-Pretoria counterparts. Therefore, most of the species tested by Miss Wing, as well as some additional plant species, were included in the no-choice, cut-leaf trials.

Adults (4 females and 2 males) were placed in round, clear, ventilated plastic containers (volume about 0.5 litre)(Figure 6) in which the eggs were laid. Neonate larvae were transferred from the containers with the use of an artist's brush, and placed onto the leaves of the different plant species. Every test plant species was replicated three times, using 6 larvae per replication. The containers (oblong, square 95x75x45mm, clear and ventilated) (see Figure 6) were checked, and the larvae provided with fresh leaves, on a daily basis. Data recorded included: mortality, feeding, date of pupation and date of adult emergence (Appendix F). *Diota rostrata* development from neonate to pupa was completed on only 12 of the 58 plant species tested (Table 8). Observations were made at ambient conditions in the laboratory.

Figure 6. Containers used for *Diota rostrata* larvae rearing (left), egg laying (center) and cut leaf trials (right).

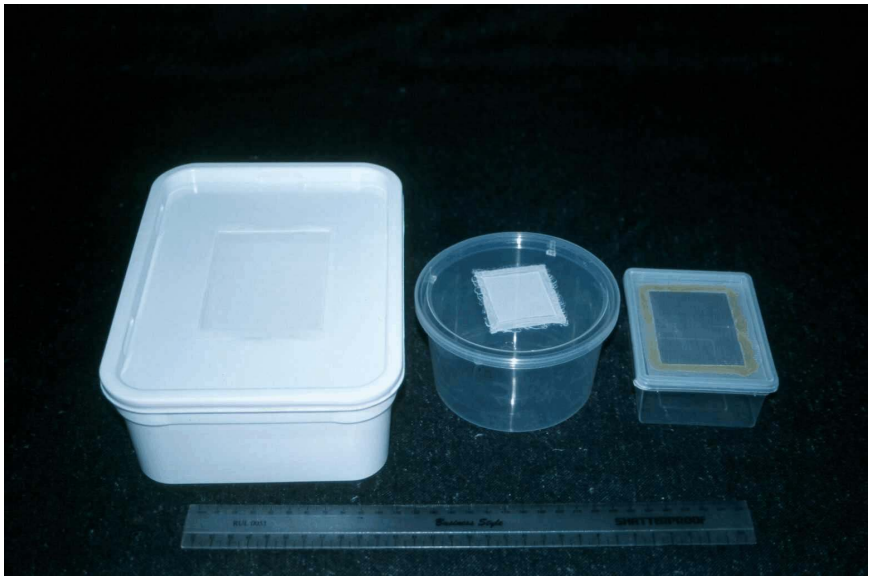


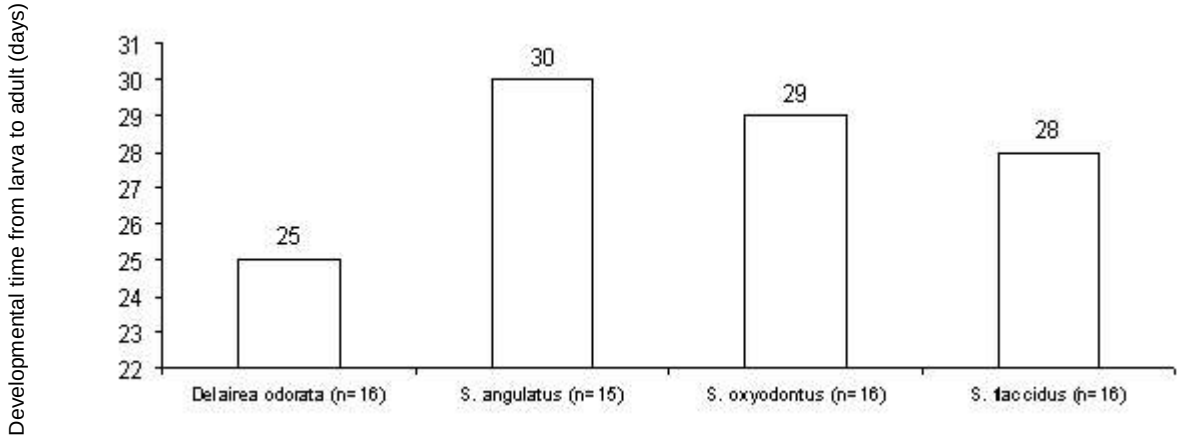
Table 8. Plant species tested as potentially suitable for complete larval development of *Diota rostrata*. Trials were replicated 3 times.

Genus and species	Common name	Family if not Asteraceae	Asteraceae Tribe	Acceptability to *D. rostrata*	Fleshiness of leaves
Same sub-tribe as Delairea odorata					
Delairea odorata	Cape ivy		Senecioneae	Yes	++
Euryops pectinatus	grey-leaved euryops		Senecioneae	No	0
E. chrysanthemoides	golden daisy		Senecioneae	No	0
Mikaniopsis cissampelina			Senecioneae	Yes	++
Senecio angulatus			Senecioneae	Yes	++
S. bolanderi (USA)			Senecioneae		
S. brachypodus			Senecioneae	Yes	++
S. deltoideus			Senecioneae	No	0
S. flaccidus (USA)			Senecioneae	Yes	+
S. glastifolius	fountain daisy		Senecioneae	No	+
S. helminthioides			Senecioneae	Yes	++
S. macroglossis	flowering ivy		Senecioneae	No	++
S. oxyodontus			Senecioneae	Yes	++
S. oxyriifolius			Senecioneae	No	++
S. pleistocephalus			Senecioneae	Yes	++
S. quinquelobus			Senecioneae	Yes	++
S. tamoides	canary creeper		Senecioneae	Yes	++
Senecio sp. (unidentified)			Senecioneae	No	++
Other Senecioneae					
Cineraria cv "butterfly"			Senecioneae	No	0
C. deltoidea			Senecioneae	No	+
C. lobata			Senecioneae	No	+
Crassocephalum crepidioides			Senecioneae	No	0
Kleinia abyssinica			Senecioneae	Yes	++
Commercially important Asteraceae					
Helianthus annuus	sunflower		Heliantheae	No	0
Helianthus tuberosus	Jerusalem artichoke		Heliantheae	No	0
Carthamus tinctorius	safflower		Cardueae	No	0
Cynara scolymus	globe artichoke		Cardueae	No	0
Cichorium intybus	chicory		Lactuceae	No	+
Gerbera jamesonii	gerbera		Mustisieae	No	0
Lactuca sativa	lettuce		Lactuceae	No	0
Commercially important non-Asteraceae					
Daucus carota	carrot	Apiaceae		No	0
Brassica oleracea	Cabbage	Brassicaceae		No	+
Raphanus sativus	radish	Brassicaceae		No	0
Beta vulgaris var. *cicla*	leaf spinach/Swiss chard	Chenopodiaceae		No	+
Rosa cv.	rose	Rosaceae		No	0

Other Asteraceae					
Bidens formosa	cosmos		Heliantheae	No	0
Calendula officinalis	calendula		Calenduleae	No	+
Centaurea cyanus	coneflower		Cardueae	No	0
Coreopsis sp. cv.	tickseed		Heliantheae	No	0
Dahlia pinnata cv	garden dahlia		Heliantheae	No	0
Felicia amelloides	blue felicia		Astereae	No	+
Rudbeckia sp. cv.	cornflower		Heliantheae	No	0
Tagetes minuta	tall khaki weed		Helenieae	No	0
Zinnia elegans cv.	zinnia		Heliantheae	No	0
Widespread weeds (mainly Asteraceae)					
Acanthospermum brasilum	Brazilian starbur		Vernonieae	No	0
Ageratina adenophora	crofton weed/Mexican devil		Eupatorieae	No	0
Ageratina riparia	mistflower		Eupatorieae	No	0
Ageratum houstonianum	Mexican ageratum		Eupatorieae	No	0
Bidens pilosa	black jack/ beggar-ticks		Helenieae	Yes	0
Chromolaena odorata	Siam weed		Eupatorieae	No	0
Flaveria bidentis	smelter's bush		Helenieae	No	0
Galinsoga parviflora	small-flowered quick weed		Helenieae	No	0
Campuloclinium macrocephalum	pompom weed		Eupatorieae	No	0
Macfadyena unguis-cati	cat's claw	Bignoniaceae		No	0

Delairea odorata was included only in the tests during the summer, making it very difficult to compare developmental time with species tested during the winter months (29 days vs. 59-88 days). Instead of using all 12 species on which *Diota* managed to pupate, only four key species were selected for this purpose (Figure 7 and Appendix E).

Figure 7. Developmental time from larva to adult of *Diota rostrata* larvae on leaves of four different host plants at ambient conditions with daily summer temperatures ranging between 21- 27°C. Trials were replicated 3 times.



Other tribes of the Asteraceae in which test plants could be available, if required:
Eremothamneae – endemic but not readily available (2 genera, 3 spp.)
Vernonieae – e.g. *Vernonia* spp.
Arctoteae – various genera, e.g. *Arctotis, Arctotheca, Gazania*
Tarchonantheae – 2 genera (trees): *Brachylaena* and *Tarchonanthus* spp.
Anthemideae – many genera e.g. *Ursinia, Chrysanthemum* and *Cotula*
Gnaphalieae – genera e.g. *Callilepis, Facelis* and *Helichrysum*
Inuleae – several genera
Plucheeae – e.g. *Pluchea* spp.

Results and discussion

Trials were done under conditions in a room experiencing roughly ambient conditions. Daily temperatures ranged form 21°C to 27°C in summer, and 14°C to 24°C in winter. Results of the key species trial indicate that *Diota rostrata* developed on some other asteraceous hosts apart from *Delairea odorata* under laboratory conditions. In contrast to work done by Miss J. Wing, these trials seem to indicate a relatively faster developmental rate on *D. odorata.* Proper statistical analysis still needs to be done before drawing any conclusions (see appendix C for descriptive analysis). Development on the three *Senecio* spp. (*S. angulatus, S. oxyodontus* and *S. tamoides*) on which the insect has been collected in the field and in gardens was to be expected however, development on *S. flaccidus, Bidens pilosa* and *Kleinia abyssinica* indicates that at lest in no-choice situations larval development was possible on a wider range of species than the recorded or natural range.

Oviposition testing

The no-choice, cut-leaf trials gave a fairly good indication as to which plant species would sustain the *Diota rostrata* larvae to pupation. Whether the female will select the same species for oviposition is a totally different matter. For this reason it was important to try and simulate conditions that would allow the female to oviposit on a plant of her choice. Results obtained from this trial will also assist in the decision with regards to the future of *Diota rostrata* as a possible biological control agent on *Delairea odorata*.

Method

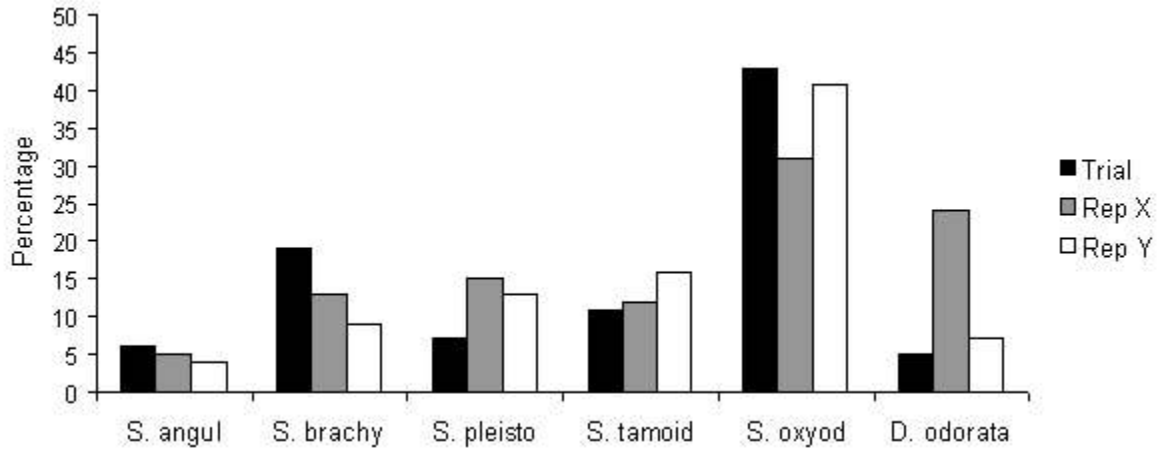
A walk-in cage (4mx4mx2m) made from "psylla screen" was used for this purpose. The trial was set up in a fiberglass tunnel with a wet wall on the southern end and an extraction fan on the northern end. The number of plants that needed to be tested and the fact that only one cage was available for the planned trial prompted the use of a lattice design instead of a Latin square (Appendix G). 14 pairs of adults were released evenly throughout the cage and egg groups as well as the number of eggs per group and the date of emergence were recorded. Test plants used included all 10 species that sustained the development of *Diota* larvae as well as 2 non-target plants.

The latter was included to have some degree of a control to indicate whether the females laid their eggs indiscriminately under the conditions used. The trial consisted of a trial run, and two replicates (The third replicate is to be completed) (Table 9 and Figure 8)

Table 9. Preliminary results of an oviposition test in a walk-in cage that contain recorded host plants and hosts unsuitable (**) for larval development

Species	Trial run (6 days)		Rep X (6 days)		Rep Y (7 days)	
	No of eggs	% of total no of eggs	No of eggs	% of total no of eggs	No of eggs	% of total no of eggs
S. angulatus	21	5.9	7	5.2	35	4.4
S. brachypodus	67	19.1	18	13.3	72	9.1
S. pleistocephalus	24	6.8	20	14.8	101	12.7
S. tamoides	37	10.5	16	11.9	125	15.7
S. helminthioides	-	-	-	-	51	6.4
S. quinquelobus	-	-	-	-	29	3.7
S. oxyodontus	150	42.7	42	31.1	322	40.6
S. flaccidus	-	-	-	-	-	-
Mikaniopsis cissampelina	35	9.9	-	-	-	-
*Cineraria lobata***	-	-	-	-	-	-
*Bidens pilosa***	-	-	-	-	-	-
Delairea odorata	17	4.8	32	23.7	59	7.4
Total	351	100	135	100	794	100

Figure 8. Number of eggs laid by *Diota rostrata* on each plant species (% of total eggs laid)



The preliminary data seem to indicate that female *Diota* moths prefer *S. oxyodontus* for oviposition to all other species used in the trial. This may be an indication that *Delairea odorata* was not the primary host of the *Diota rostrata* population used in the trial ("Kirstenbosch culture"). A more detailed analysis will be put forward as soon as the final replicate has been completed. It is interesting that although larvae had been shown to be able to develop on

Biddens pilosa (not a known host is the field), no eggs were laid on this species in the experiment. It is also envisaged to repeat the trial with moths from specifically *D. odorata* and from another area where the insects occurs naturally on another host.

5. Leaf Beetles *Liperodes* cf *tibialis* (Coleoptera: Chrysomelidae)

No new adults of the Galerucine chrysomelids of which the larvae may be root feeders, were reared in the ensuing year from potted plants exposed late 2000, over a long period (until all the adult beetles disappeared) to actively feeding adults. It is possible that *Delairea odorata* may not be a larval host of these beetles, and further observations will have to be made near the only site where adults have been collected, if work on this beetle is to be pursued. Keeping exposed plants in captivity in a healthy condition for such a long period, without the possibility of safely

III. Yellow starthistle research

A. Introduction

Between 1996-2000, yellow starthistle (YST), *Centaurea solstitialis*, was the primary focus for research by Dr. Balciunas. In late 2000, Dr. Link Smith joined our research group in Albany, and during 2001, the leadership of the biological control of yellow starthistle project was transferred to him. This portion of our report covers the final stages of yellow starthistle research, primarily focused on *Chaetorellia succinea*, headed by Dr. Balciunas.

Yellow starthistle is an annual weed native to the eastern Mediterranean region of Eurasia. It was introduced into California more than 150 years ago (Maddox and Mayfield 1985). It is now the state's worst weed, and is also causing severe problems in parts of Oregon, Washington, and Idaho. Rangelands infested with YST are unproductive due to disruption of grazing by this weed's sharp spines and a neurologic disorder produced in horses if digested (Cordy 1978). In California, the area infested increased from an estimated 1.2 million acres in 1958 to 7.9 million acres in 1985 (Maddox and Mayfield 1985). YST's logarithmic range expansion continues. A 1997 survey by California Department of Food and Agriculture (CDFA) found this weed in 42 % (n = 1,935) of California's 4,638 townships - each six by six mi. square - and in 22 % (1,019 townships) the infestations are reported as "high" (Pitcairn *et al.* 1998). "High" abundance was defined as being, at a minimum, several miles of dense roadside infestation.

Overseas surveys to locate potential biocontrol agents for YST began in Europe 40 years ago, and to date seven insect species have been released in the United States for control of this invasive weed (Table 10), all of which attack the flowers or seeds of YST.

Table 10. A list of agents imported and intentionally released in the U.S., for biocontrol of yellow starthistle.

Biocontrol agent	Date of release	Status
Urophora jaculata (Diptera: Tephritidae)	1969	Never established in North America
Urophora sirunaseva (Diptera: Tephritidae)	1984	Widely established, present at most YST infestations. Minimal impact on YST.
Bangasternus orientalis (Coleoptera: Curculionidae)	1985	Widely established, present at most YST infestations. Minimal impact on YST.
Chaetorellia australis (Diptera: Tephritidae)	1988	Established, but only at locations where *Cnt. cyanus* is also present. Minimal impact on YST.
Eustenopus villosus (Coleoptera: Curculionidae)	1990	Well established, being redistributed. Some localized reductions of YST populations.
Chaetorellia succinea (Diptera: Tephritidae)	1991	Accidentally introduced, well established in CA and parts of NV. May be reducing YST populations.
Larinus curtus (Coleoptera: Curculionidae)	1992	Established at a few release sites in CA, OR, WA, and ID. Limited impact.

The first insect *Urophora jaculata*, released in 1969, never established. Fifteen years later *Urophora sirunaseva* - a gall fly - was released, and *Bangasternus orientalis* - a seed head weevil - one year after that. Both are well established, but are not causing much damage to YST. Two more seed head weevils - *Eustenopus* (*Eu.*) *villosus* and *Larinus* (*Lr.*) *curtus* were released in the early 1990s. We studied *Lr. curtus* in 1999 and its lack of impact was discussed in our earlier report (Balciunas *et al.* 2000). *Eu. villosus* appears to be causing localized YST reductions at sites in Washington, Oregon, Idaho, and California.

The fourth insect to be approved and released in the U.S. for control of YST was the seed head fly *Chaetorellia* (*Ch.*) *australis*. This fly's larvae feed inside the seed heads of YST, destroying most of the developing seeds. Mature larvae overwinter in old heads, and the adults emerge in the spring. Females oviposit on maturing buds, and several generations are completed before winter. Releases of *Ch. australis*, reared from YST heads shipped from Greece, began in 1988. By 1994, this fly had been released at 14 sites in California, Idaho, Oregon, and Washington. However, establishment was confirmed only at one site each in Oregon and Washington, and in 1995, at one of the Idaho sites (Turner *et al.* 1996). Establishment of this fly was not observed at any of the six California sites (Turner *et al.* 1996). At the three sites (in Idaho, Oregon, and Washington) where it did establish, *Centaurea* (*Cnt.*) *cyanus*, (bachelor's button) was widespread. *Cnt. cyanus* is another exotic annual, closely related to YST, which is invasive in the Pacific Northwest. It was theorized that the early-blooming *Cnt. cyanus* flowers were acting as an alternate host until YST blossomed some weeks later (Turner *et al.* 1996).

B. Discovery of the unintentionally-introduced *Chaetorellia succinea*

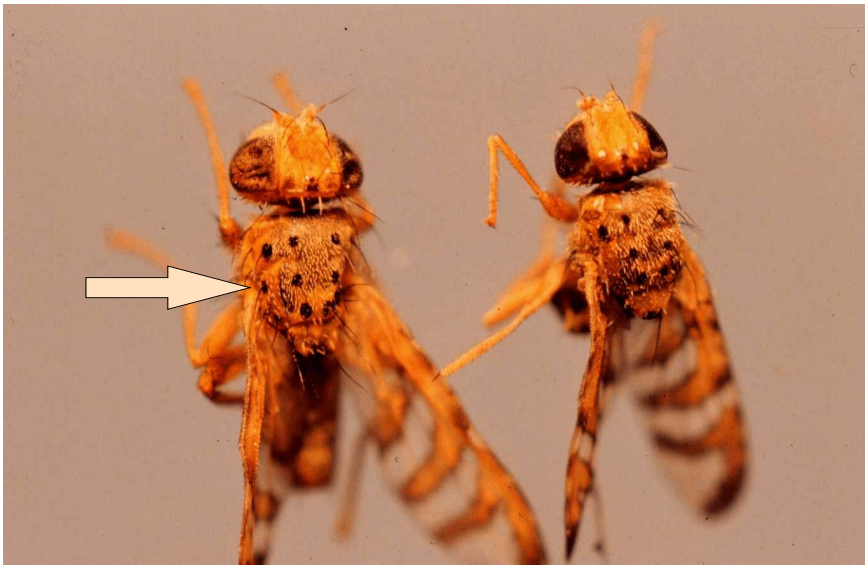
Buoyed by these successful establishments, with the assistance of the CDFA, the colonization effort for *Ch. australis* in California was renewed, with releases at seven sites in seven counties during 1995, and a further 15 releases in 12 counties during 1996. Sites containing both *Cnt. cyanus* and YST were given the highest priority, and second priority was given to sites with early blooming YST. All flies released were those that emerged from YST heads (except one sample from *Cnt. cyanus*) collected at the Merlin, Oregon site. During CDFA's surveys at the end of 1995, populations of this fly were found at multiple locations in Humboldt and Trinity Counties in northern California. The populations in these counties were so large and wide-spread, that we surmised that they were the result of natural migration from the long-established populations at the Merlin, Oregon release site (107 mi. away), rather than from our releases earlier that year in Shasta and Siskiyou Counties. By late 1996, flies were recovered from all of the 1995-96 release sites, indicating at least temporary establishment.

The ease with which these flies from Oregon established at all sites, including those that lacked *Cnt. cyanus*, along with their rapid dispersal from the release sites, was unexpected – especially in light of the complete failure of the earlier releases in California. Specimens of the flies recovered from the field in California were submitted to two experts on fly taxonomy at the CDFA Plant Pest Diagnostics Center. Neither Dr. Louie Blanc nor Dr. Eric Fisher thought that these California flies fit the published description of *Ch. australis*, and Dr. Fisher identified them

as either *Ch. succinea*, a similar species from Europe and Asia or *Ch. carthami*, an incidental pest of safflower in the Middle East.

After receiving these preliminary identifications, all further releases of *Chaetorellia* flies in California were immediately curtailed, due to the potential negative environmental effects of this accidental introduction. We assembled *Chaetorellia* specimens recovered from field sites in California, Oregon, and Washington, and shipped these, along with voucher *Chaetorellia* specimens from those originally imported and tested at the ARS quarantine in Albany, Dr. Ian White at the British Museum of Natural History, London for confirmation. Dr. White is an authority for the genus *Chaetorellia*, and had recently published a revision of this genus (White and Marquardt 1989). He confirmed that the majority of *Chaetorellia* specimens from California and Merlin, Oregon were, in fact, *Ch. succinea*. White and Marquardt (1989) place the nine known species of *Chaetorellia* into two groups, with *Ch. succinea* belonging to the *Ch. loricata* group, and *Ch. australis* to the *Ch. jaceae* group. *Ch. succinea* (and the other two species of the *Ch. loricata* group) each have an extra "spot" on each side of its thorax that is lacking in *Ch. australis* and the other five species in its group. Since no other members of the *Ch. loricata* have been recorded in North America, we use this extra "spot" (shown in Figure 9) as an easy way to distinguish it from all other *Chaetorellia* flies found here. Additional details on the discovery and accidental release of this fly are presented in Balciunas and Villegas (1999).

Figure 9. A photo of the extra thoratic "spot" on *Chaetorellia succinea* (on left) as compared to *Chaetorellia australis* (on the right).



At the end of 1998, the results of the surveys in California showed that *Ch. australis* has established in a few scattered release sites, all of which had *Cnt. cyanus* in addition to YST. On the other hand, *Ch. succinea* is now well established and spreading rapidly. It is widespread from southwestern Oregon to as far south as Stockton, California, and we also recovered this fly from several sites around Reno, Nevada.

C. *Chaetorellia succinea* laboratory host range research

We have studied the host range of the unintentionally introduced fly, *Chaetorellia* (*Ch.*) *succinea* for the last six years, both to ascertain which hosts it was using [or ignoring] in the field, and obtaining laboratory results to confirm lack of attack, and to predict additional field hosts.

1. Laboratory no-choice and choice tests

We continued to evaluate the host range of *Ch. succinea* on several native and exotic Cardueae plants at our quarantine greenhouse facilities in Albany through March 2002. These tests were conducted primarily using *Ch. succinea* that emerged from yellow starthistle heads collected at Wildcat Canyon, and occasionally *Ch. succinea* from other sites in California and Nevada.

We tested these newly emerged flies (1-3 days old) for oviposition and development by confining them in sleeve cages (73 x 42 x 449 cm) or screen cages (122 x 91.5 x 91.5 cm) to Cardueae plants. Plants tested each had at least one, but usually several, mature closed heads appropriate for oviposition and development. For the duration of each test, confined flies were supplied a nutrient source of 50 % Mountain Dew © soda (Coca-Cola© Company). Tests were run for 14-21 days to allow sufficient time for *Ch. succinea* oviposition, and development on Cardueae heads.

After the tests were completed, flies were removed and the plants were kept alive for at least three weeks to allow *Ch. succinea* to develop, and were monitored for adult emergence. The heads were removed and kept for 1-2 more weeks, then dissected to verify the presence or absence of *Ch. succinea*. Voucher specimens are kept at the USDA-WRRC. Results from these no-choice tests are shown in Table 11.

Table 11. Larval infestation rates to test plants in the tribe Cardueae and paired yellow starthistle controls exposed to *Chaetorellia succinea* adults under no-choice conditions.

Test		*Ch. succinea*		Test Plant				Yellow starthistle Control				
Test No.	Test duration (days)	Population	n	Species	Total heads	No. of heads infested by *Ch. succinea*	Infested heads /	n	Total heads	No. of heads infested by *Ch. succinea*	Infested heads /	Fisher Exact test two tailed
CH-26-99	22	WC	5	*Carthamus baeticus* (Boiss. & Reuter) Nyman	8	0	0	4	15	10	2.5	<.001***
CH-31-99	22	WC	10	*Carthamus baeticus*	22	0	0	6	13	7	1.17	<.001***
CH-6-01	14	WC	3	*Centaurea americana* Nutt.	4	1	0.33	5[b]	17	6	1.2	.503

CH-7-01	14	WC	4	*Centaurea americana*	5	2	0.5	2	6	4	2.0	.105
CH-8-01	14	WC	6	*Centaurea americana*	3	0	0	5	10	7	1.4	.009**
CH-20-99	21	RB	5	*Centaurea calcitrapa* L.	48	0	0	5	38	11	2.2	<.001***
CH-12-00	14	Var.	8	*Centaurea cyanus* L.	30	0	0	4	12	7	1.75	<.001***
CH-14-00	14	SB	10	*Centaurea cyanus*	38	0	0	6	13	5	0.83	<.001***
CH-19-01	14	WC	3	*Centaurea diffusa* Lam.	34	0	0	2	19	3	1.5	<.001***
CH-10-00	14	Laf.	5	*Centaurea maculosa* Lam.	10	0	0	3	6	4	1.33	<.001***
CH-9-01	14	WC	4	*Centaurea melitensis* L.	46	7	1.75	2	12	4	2.0	.006**
CH-10-01	14	WC	6	*Centaurea melitensis*	51	2	0.33	3[b]	24	8	2.67	<.001***
CH-15-01	14	WC	6	*Centaurea melitensis*	28	3	0.5	1	12	4	4.0	.002**
CH-2-01	14	WC	12	*Centaurea rothrockii* Greenm.	4	0	0	2	25	14	7.0	.090
CH-2-02	14	WC	6	*Centaurea rothrockii*	3	0	0	4	15	10	11.1	.069
CH-1-00	21	WC	10	*Centaurea sulphurea* Willd.	12	4	0.4	9	10	5	0.56	.680
CH-3-00	21	Ione	10	*Centaurea sulphurea*	8	2	0.2	6	29	14	2.33	.277
CH-6-00	21	Ione	6	*Centaurea sulphurea*	6	0	0	6	12	8	1.33	<.001***
CH-5-96	63	RC	12	*Cirsium brevistylum* Cronq.	38	0	0	12[c]	274	113	9.42	<.001***
CH-1-99	35	NV	9	*Cirsium brevistylum*	6	0	0	4	9	4	1.0	.064
CH-11-00	14	Laf.	8	*Cirsium brevistylum*	3	0	0	7	17	9	1.29	.102
CH-3-01	14	WC	5	*Cirsium hydrophilum* var. *vaseyi* (A. Gray) J. Howell	6	0	0	3	11	2	0.67	.171
CH-7-00	14	Ione	6	*Cirsium occidentale* var. *candidissimum* (E. Greene) J.F. Macbr.	5	0	0	2	13	4	2.0	.097
CH-16-00	14	Var.	6	*Cirsium occidentale* var. *candidissimum*	3	0	0	5	23	8	1.6	.167
CH-5-00	21	Ione	3	*Cirsium ochrocentrum* A. Gray	1	0	0	2	11	2	1.0	1.000
CH-17-00	14	WC	4	*Cirsium ochrocentrum*	1	0	0	2	19	7	3.5	.551
CH-30-99	21	WC	6	*Silybum marianum* (L.) Gaertner	5	0	0	5	17	5	1.0	.165
CH-32-99	21	WC	7	*Silybum marianum*	3	0	0	4	17	8	2.0	.126

[a] *Ch. succinea* populations: (all reared from yellow starthistle except RC, flies swept from yellow starthistle) RC - Rancho Cordova, Sacramento County, CA. NV - Washoe Co., Nevada. RB - Red Bluff, Tehama Co., CA. WC - Wildcat Canyon, Contra Costa Co., CA. Ione - Ione, Amador Co., CA. Laf. - Lafayette, Contra Costa Co., CA. Var. - Various, multiple locations of the previous six sites, CA. SB - Sutter's Butte, Butte Co. CA.

[b] In the CH-5-96 test, the YST control test was run simultaneously with *Ch. succinea* no-choice oviposition / development tests on *Cir. brevistylum* using different flies. Consequent tests used flies surviving no-choice oviposition / development tests in post YST control tests.

[c] No female *Ch. succinea* adults survived the test plant portion of the test. Yellow starthistle control data was derived from pooling yellow starthistle control data from each test run before and after the test without a yellow starthistle control.

** $P < 0.01$, *** $P < 0.001$; Fisher's exact test of proportion of infested vs. non-infested heads per female*10.

Under no-choice conditions, *Ch. succinea* indicated a larger host range than we expected. It oviposited and developed on the introduced *Centaurea* (*Cnt.*) *sulphurea*, and *Cnt. melitensis*, and the native *Cnt. americana*.

The three test plant species that were attacked by *Chaetorellia succinea* during the no-choice tests, were then evaluated under choice conditions. These choice tests were similar to no-choice tests, except that flies were simultaneously exposed to yellow starthistle and test plant species in the same cage. Table 12 presents the results of choice tests on the three species of *Centaurea* infested with *Ch. succinea* in the earlier no-choice tests.

Table 12. Larval infestation rates to test plants in the tribe Cardueae and paired yellow starthistle controls exposed for 14 days to six pairs of *Chaetorellia succinea* adults under choice conditions.

Test	Test Plant			Yellow starthistle control		
Test No.	Species	Total Heads	No. of heads infested by *Ch. succinea*	Total heads	No. of heads infested by *Ch. succinea*	Fisher Exact test two tailed *P* value
CH-12-01	*Centaurea americana*	7	0	10	1	1.000
CH-20-01	*Centaurea americana*	6	0	27	6	.563
CH-4-02	*Centaurea americana*	17	1	21	13	<.001***
CH-9-02	*Centaurea americana*	13	1	19	5	.361
CH-13-01	*Centaurea melitensis*	35	0	17	4	.009**
CH-14-01	*Centaurea melitensis*	50	6	18	1	.666
CH-5-02	*Centaurea melitensis*	76	1	23	16	<.001***
CH-16-01[a]	*Centaurea sulphurea*	9	0	30	18	.002**
CH-17-01	*Centaurea sulphurea*	10	0	14	2	.493
CH-18-01	*Centaurea sulphurea*	7	1	24	12	.191

Ch. succinea from Wildcat Canyon, Contra Costa Co., CA.

[a] Four pairs of *Ch. succinea* used in this test rather than six.

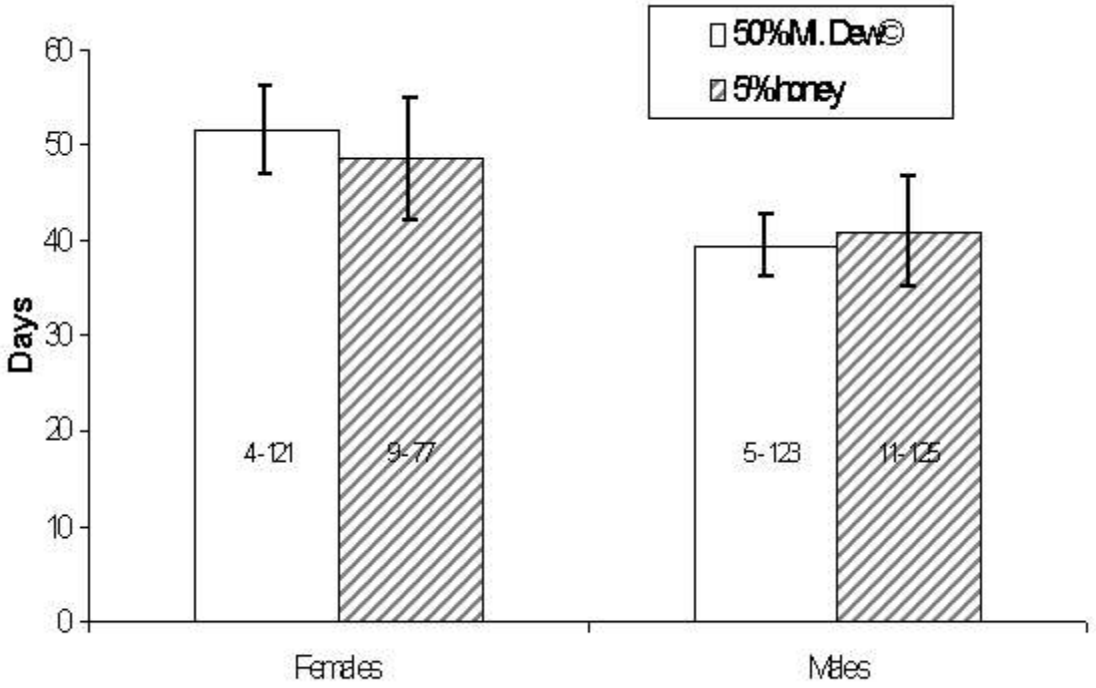
** $P < 0.01$, *** $P < 0.001$; Fisher's exact test of proportion of infested vs. non-infested heads.

Oviposition was observed on all three species, even under choice conditions, but in only half the tests. *Cnt. americana* is one of two members of *Centaurea* that are native to North America. Currently, *Cnt. solstitialis* and *Cnt. americana* are isolated from each other, but *Cnt. melitensis* overlaps the native ranges of both. We are concerned that *Cnt. melitensis* may serve as a stepping stone host that might allow *Ch. succinea* to reach *Cnt. americana*.

2. Longevity tests

Little is known about the biology and life history of the accidentally introduced *Chaetorellia succinea*. In addition to our host range experiments, we attempted to determine the average survival rate of males and females kept at a constant temperature (20 °C) in a Conviron model No. E7 growth chamber with a regular regime of light (16:8 light:dark).

Male and female pairs of newly emerged (within one day) *Ch. succinea* adults were kept in plexiglass tubes (26cm height, 14.5cm diameter) covered with plastic mesh. A nutrient source, either 50% Mt. Dew© soda and 50% water, or a 5% honey (with a small amount of yeast hydrolysate) and 95% water, source was supplied to the flies as needed. Except for weekends and holidays, these longevity tests were checked daily. Dead flies were removed, kept as voucher



specimens, and replaced with another fly of the same sex. Results are shown in Figure 10.

Figure 10. Longevity of *Chaetorellia succinea* adults kept in an environmental chamber (20°C, 16:8 L:D) with two different nutrient sources. Bars represent standard error, values inside bars represent the range of longevity in days.

Although female flies lived longer than males in both treatments, only the Mt. Dew© treatment was statistically different: Student's T-test - $t=2.167$, $df=63$, $p=.034$).

D. Field research

1. Field surveys of non-target hosts

For the past four years, in a cooperative project with CDFA, we have surveyed native and exotic Cardueae plants in parts of California and Oregon in response to concerns that *Ch. succinea* – an unintentionally introduced biological control agent for yellow starthistle – may attack these plants. Earlier, we found *Ch. succinea* infesting *Carthamus tinctorius* (safflower). We and our cooperators continued this survey through 2001. A comprehensive list of the sites and plant species that we surveyed can be found in the Appendices section - Appendix D. We have now surveyed 25 species of Cardueae plants at more than 100 sites in 33 counties in Oregon and California. We have not found *Ch. succinea* flies on any plant we surveyed, except *Cnt. melitensis* - a plant closely related to yellow starthistle.

2. Studies at Wildcat Canyon

In 1999, a study at Wildcat Canyon Park of the East Bay Regional Park District was initiated to obtain data on the phenology of YST, how it relates to the biology of *Ch. succinea*, and to monitor the impact of *Ch. succinea* on YST. Wildcat Canyon was selected as a site due to the abundance of YST and *Ch. succinea* there, as well as the site's close proximity to our laboratory (about a 15 minute drive). Vehicular access is restricted to a few authorized vehicles [including ours]. A small patch (10-20 m^2) was selected at the bottom of a hill, out of view from the trail above it. The presence of *Ch. succinea* at this site was confirmed in YST collections made in previous years.

Collections of yellow starthistle plants in Wildcat Canyon Park were made during the summer and fall of 1999, 2000, and 2001.

We occasionally visited the site to check on YST growth and development between late winter to early summer. When yellow starthistle plants began to develop mature heads in mid-July, we taking samples about every 10 days. Samples were taken by removing all YST plants from within a 0.05 m^2 circle; seven of these 0.05 m^2 circles were collected on each trip (eight during 2001). The circles were selected along a transect. The YST collected from the samples were subsequently taken back to the laboratory, where we measured the plants and then removed and stored the seedheads them in emergence containers, which were monitored daily for new *Ch. succinea* emergence. The remaining portions of the YST plant were then discarded.

The height of each YST plant and the number of plants in each circle were recorded, and the seedheads were removed, counted and were further classified according to the Maddox (1981) head stage scheme (Figure 11). We added two additional age grades (or "head stages") to the Maddox scheme: senesced heads (SH), which were essentially F2 heads whose florets had begun to turn brown, and (for the 2001 collections only), aborted heads (AH), which were immature heads that would never mature (i.e. Bu1, Bu2, or Bu3 heads that have turned brown).

Figure 11. Stages of development of heads of yellow starthistle (Maddox 1981).

The seedheads from seven circles were segregated into 250 mL and 500 mL Dixie© cups with clear plastic lids according to collection date, circle number within the collection, as well as the head age grade to await *Ch. succinea* emergence. During 2001, the YST seedheads from the eighth circle were dissected within a few days of collection to give us immediate feedback on the approximate *Chaetorellia* infestation rates at the time.

With the exception of weekends and holidays, we checked the heads stored in the Dixie cups daily for *Ch. succinea* emergence, and recorded the date and sex of all emerging flies. After emergence finished, all the heads from all the circles were dissected to detect mortality of unemerged *Ch. succinea*. Insects, larvae, or pupae present within these heads were recorded according to each head's age grade.

Table 13 on the next page shows the YST data from three years of collections. YST density in 1999 per square meter ranged between 366 and 1052 YST plants per square meter, with a grand mean density (this includes all plants in each collection) of 651.1 plants per square meter. During 2000, YST density was lower in range (386 to 680 plants/m^2) and grand mean density (519 plants/m^2). In 2001, YST density ranged between 295 to 707 plants per square meter, with a grand mean of 485.5 plants per square meter.

The mean YST plant height per collection ranged between 35 and 57 cm in 1999, 44 and 73 cm in 2000, and 45 and 60 cm in 2001. As expected, the mean plant height tended to increase as the season advanced.

The average number of seedheads per plant varied between 1.9 and 6.8 during our 1999 collections, but didn't show much of a trend. In 2000, the range was between 0.4 and 7.7 and in 2001, was between 2.1 and 11.7 seedheads per plant. In both 2000 and 2001, a trend displayed the average number of heads per plant to increase as the growing season progressed.

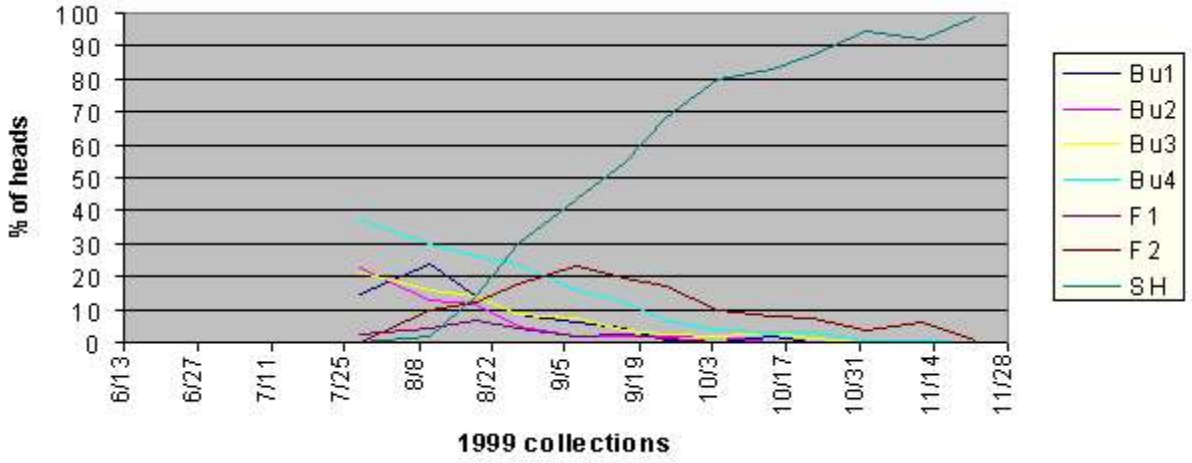
The mean seedhead density in 1999 fell between 1200 and 4140 seedheads per square meter, while in 2000 it ranged from 194 to 3882 seedheads per square meter, and 2001 it was between 677 and 6417 seedheads per square meter. For the three years, as the season progressed, the density increased.

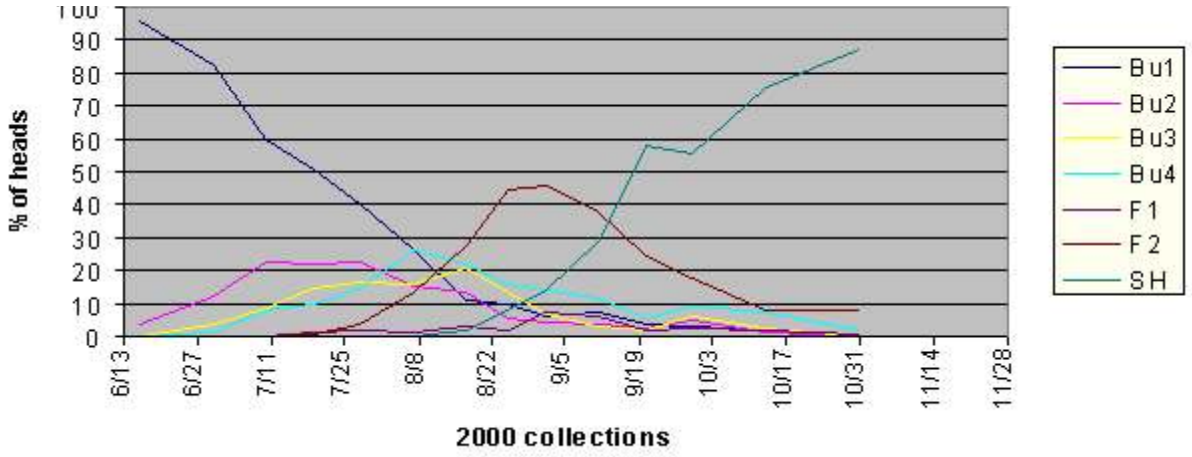
Table 13. Changes in yellow starthistle densities at Wildcat Canyon over three years (1999-2001)

Date of collection	Avg. no. of seedheads/plant	Mean plant height (cm)	Mean plant density (plants/m^2)	Mean seedhead density (seedheads/m^2)
7/21/99	3.3	37	366	1200
7/28/99	1.9	35	1052	1460
8/10/99	6.2	50	522	3254
8/19/99	4.6	46	617	2820
8/27/99	5.1	49	577	2925
9/7/99	6.8	54	482	3294
9/16/99	5.1	47	705	3577
9/24/99	3.6	50	680	2457
10/4/99	4.7	52	751	3551
10/14/99	6.0	53	594	3551
10/22/99	5.2	49	797	4140
11/1/99	4.5	52	651	2917
11/12/99	6.0	57	617	3702
11/22/99	4.6	56	700	3217
6/16/00	0.4	44	520	194
6/30/00	1.5	61	548	831
7/10/00	2.6	61	471	1242
7/19/00	4.7	73	574	2677
7/28/00	4.2	64	480	1997
8/7/00	4.6	70	517	2394
8/17/00	5.3	67	454	2400
8/25/00	7.7	67	385	2962
9/1/00	5.7	67	531	3042
9/11/00	5.2	60	680	3531
9/20/00	5.4	65	597	3234
9/29/00	7.6	64	508	3882
10/13/00	7.4	64	471	3511
10/31/00	6.7	71	525	3517
7/16/01	2.1	46	325	677
7/27/01	2.1	52	408	851
8/10/01	4.4	50	502	2200
8/23/01	3.2	52	612	1975
9/6/01	4.0	50	630	2525
9/14/01	2.8	45	707	1947
9/24/01	7.5	56	497	3732
10/4/01	10.6	57	385	4070
10/17/01	11.1	55	572	6417

10/31/01	9.8	60	407	3990
11/14/01	11.7	56	295	3445

Figure 12 shows the number of seedheads of each head stage per square meter at each collection date. As expected, as the season progressed, so did the numbers of maturing seedheads. The final collections of each year are predominantly senesced heads.





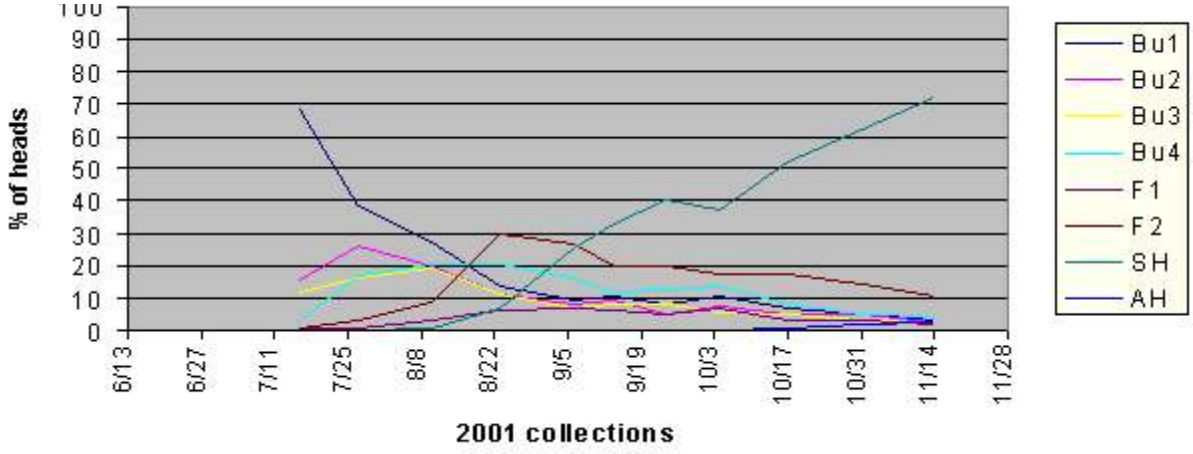
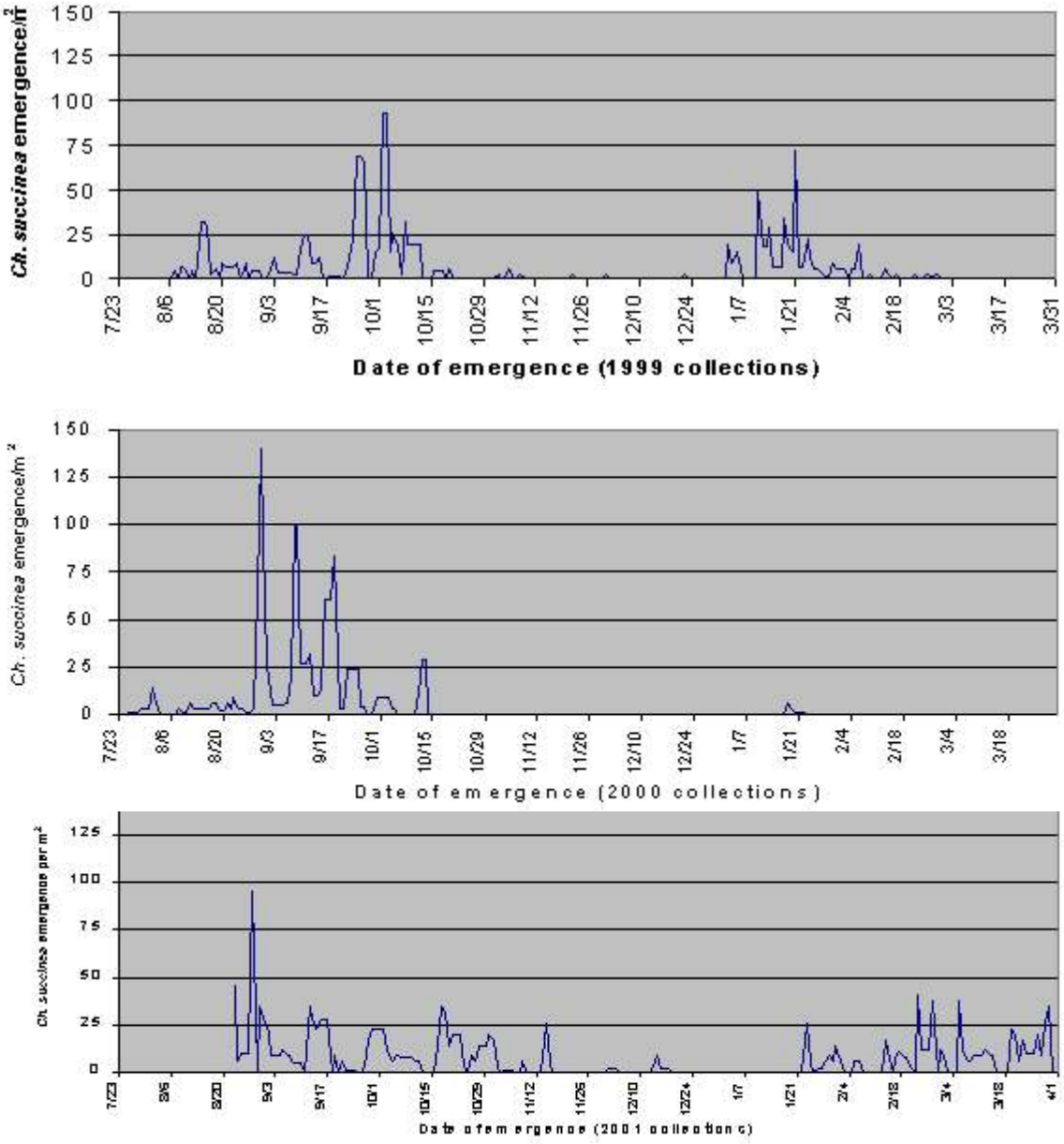


Figure 12. Changes in stages of YST seedheads per square meter at Wildcat Canyon over three growing seasons (1999-2001).

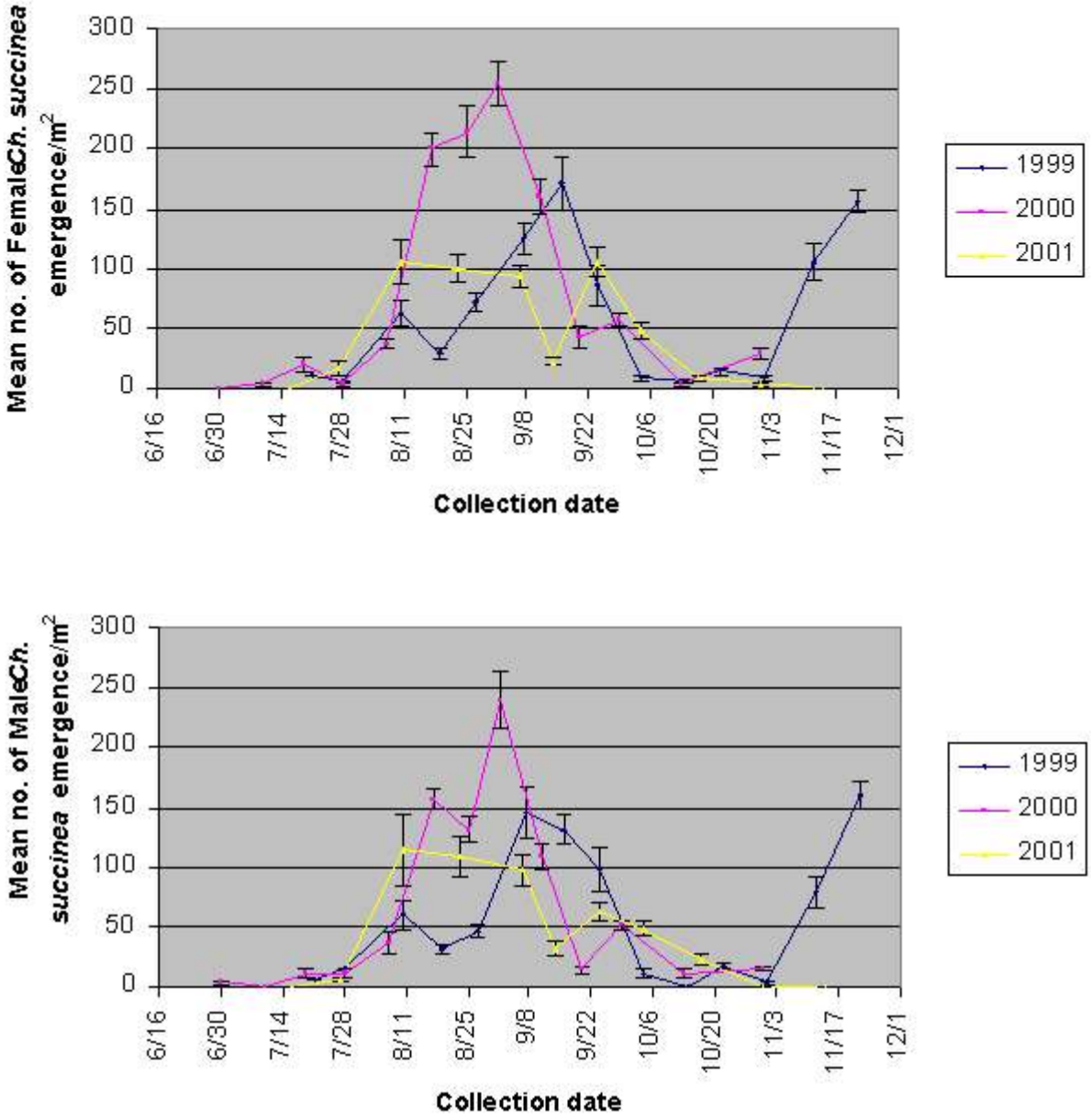
To adjust fly emergence data for weekends and holidays (when emergence was not recorded), we divided the number of flies emerging by the number of days since the previous count to arrive at a mean no. of flies per day. Figure 13 tracks *Ch. succinea* emergence per day for year of sampling. Note that the 2001 emergence data is incomplete, as it is likely that more *Ch. succinea* flies will still emerge.

Figure 13. Daily adult *Chaetorellia succinea* emergence per square meter from three years (1999-2001) of yellow starthistle collections at Wildcat Canyon.



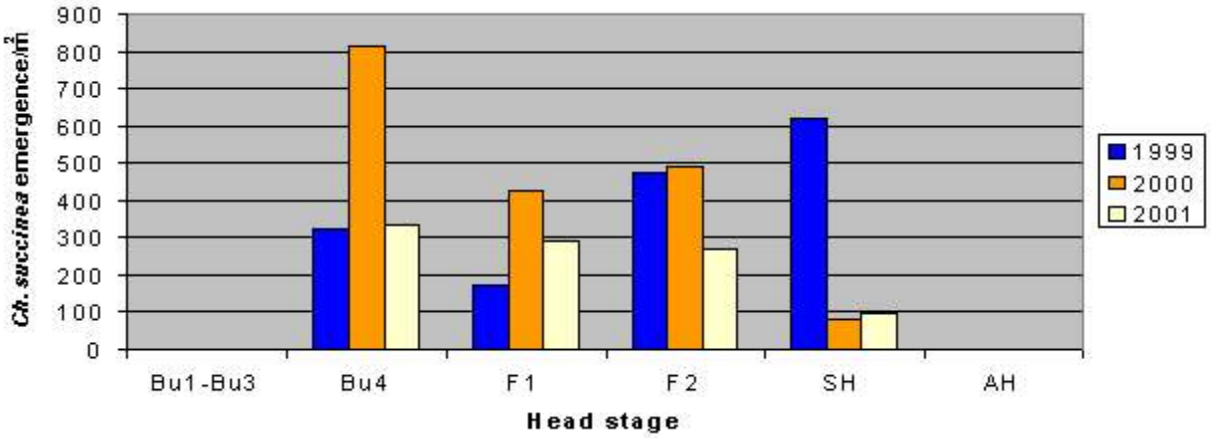
For each collection, the number of female and male *Chaetorellia* emergences from each circle were recorded, and the mean number of flies emerging per circle for both sexes was computed and converted to m^2. We plotted the means emergence per square meter of both females and males with standard error bars for all collections from 1999-2001 (Figure 14). Some data for dead *Ch. succinea* larvae and pupa are plotted. Note that the 2001 emergence data is incomplete, as *Ch. succinea* flies are likely to emerge in the next few months.

Figure 14. The mean female and male *Chaetorellia succinea* emergence per square meter for collections from 1999 through March 2002. Error bars indicate the standard error.



We also wanted to see how the phenology of YST related to *Ch. succinea* emergence. Figure 15 shows the amount of *Ch. succinea* emergence per square meter for each head stage.

Figure 15. Emergence of adult *Chaetorellia succinea* emergence per square meter from different head stages of YST collected at Wildcat Canyon.

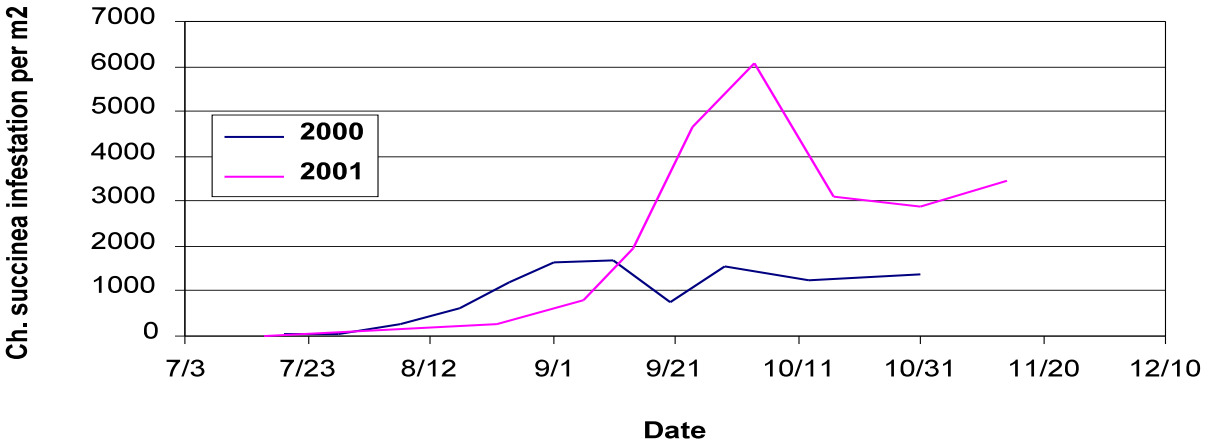


No flies emerged from YST head stages Bu1, Bu2, Bu3, or, in 2001, AH. Interestingly, the linear trend in the 1999 collection is opposite that of the following two years' collections. In 1999, the greatest emergence originated from Bu4 heads, while the least emergence came from SH heads. In the 2000 and 2001 collections, the reverse is true.

We suspected that *Ch. succinea* were dying in our laboratory stored heads, rather than emerging. We dissected some old heads from 1999 collections. We were surprised to discover that the vast majority of *Ch. succinea* in these heads never emerged as adults. They died in their larval stages inside the seedheads. The numbers of *Ch. succinea* that emerged in our laboratory were dwarfed by the larval deaths. Overall, in our laboratory, only 15% of the larvae that we collected from the field, emerged successfully.

We then calculated the *Ch. succinea* infestation rate, which is the sum of *Ch. succinea* larval deaths and emergences per square meter. Figure 16 shows the infestation rate at Wildcat Canyon for 2000 and 2001.

Figure 16. 2000-2001 *Ch. succinea* infestation rate per square meter at Wildcat Canyon.



Thus, when dead larvae are included, the infestation rate for 2001 was more than three times that of 2000.

3. Wildcat Canyon seed bank studies

Throughout 2001 we periodically collected soil cores from the Wildcat Canyon study site to determine the number of YST seeds in the "seed bank" at various times throughout the year. The soil cores were collected along the 2001 transect as were the YST collections. The soil cores were collected with an Oakfield soil corer (Oakfield Apparatus, Inc., Oakfield, WI) by taking a soil core at one step intervals. The area of the soil corer is 2.27×10^{-4} m^2. Approximately 20 cores were taken at a time. Soil cores were brought back to our laboratory and soaked with water to break up soil. The resulting solution was examined for YST seeds, which were recovered and dried. These recovered seeds were planted to obtain germination and confirm that the seeds recovered were YST seeds.

Figure 17 shows the collection dates for soil core extractions and the number of YST seeds found per square meter. The maximum density was approximately 15,000 seeds per square meter in mid-February 2000.

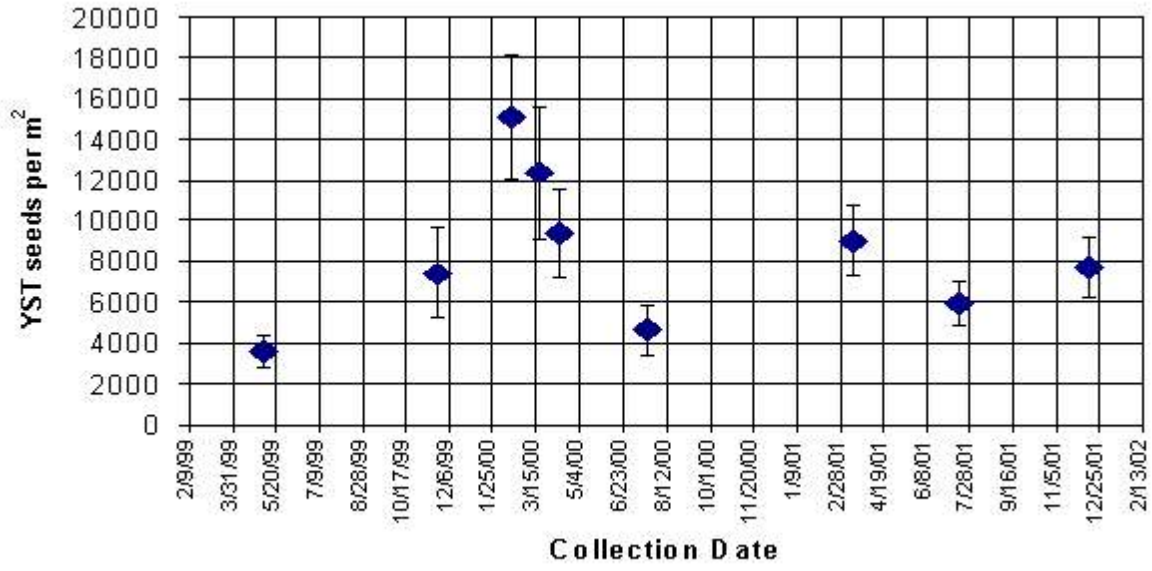


Figure 17. Seed densities in semi-annual soil core samples (1999-2001) at Wildcat Canyon. Error bars indicate the standard error.

Generally, the density of YST seeds was lowest during the summer months and highest in the early months of the new year when YST skeletons began dropping their seedheads.

IV. Other activities and publications

A. Publications issued or submitted

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Balciunas, J. 2001. Test plants needed for biocontrol of Cape ivy project. CalEPPC News. 9: 4-6.

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B. Selected meetings and travel by Dr. Joe Balciunas

2001 Meetings, Travel, & Training

Jan 9	South Africa	return from 1 month trip reviewing PPRI research, and planning Year 4 research
Jan 22	Albany	Attend Quarantine Committee Meeting
Jan 24	Albany	Recertification training for "Red Cross First Aid"
Feb 9-11	Joshua Tree	Represent ARS at CalEPPC Board meeting at Joshua Tree National Monument, CA
Feb 14	San Pablo	Serve as judge at Contra Costa County Science Fair
Feb. 15	Ft. Cronkite	collect viable Cape ivy seeds with Ellen Hamingson and Mona Robison
Mar. 5	Carmel	inspect Cape ivy at Garrapata Creek, Rio Pedros reserve, and Carmel Highlands.
Mar. 5-6	Monterey	present invited talk on biological control of weeds at CA Fish & Game Annual Weed Training Course
Mar. 14-16	Coeur d'Alene, Idaho	Present poster on *Chaetorellia succinea* at 1st International Knapweed Symposium of the 21st Century
Mar. 18	Mill Valley	Lead Weed/Native Hike #2 on the Dipsea Trail to Stinson Beach for the San Francisco Bay Sierra Club

Mar. 29	San Francisco	Serve as judge at the San Francisco Bay Regional Science Fair
Apr. 3	Davis	represent ARS at Cal EPPC Board meeting
Apr. 10	Davis	attend seminar by Plant Ecologist position candidate, Anna Sher
April 12	Meadowview	represent ARS at CINWCC meeting
Apr. 17	Davis	attend seminar by Plant Ecologist position candidate, Carla D'Antonio
Apr. 27-29	Camp Roberts	Complete Jepson Herbarium training course on "Flora of Camp Roberts"
April 30	San Luis Obispo	Present invited, 1-hr lecture on "Biological Control of Cape Ivy" to Weed Science class at CalPoly
May 1-2	San Luis Obispo	collect *Senecio* relatives in San Luis Obispo vicinity
May 5-6	Berkeley	Complete Jepson Herbarium training course on "Poaceae"
May 13	Pt. Reyes	Lead Weed/Native Hike #3 to Sculptured Beach for the San Francisco Bay Sierra Club
May 21-22	Carmel	Collect Cape ivy samples for pre-release surveys of herbivores at proposed release sites in Rio Pedros Reserve and Carmel Highlands
May 28-30	Pistol River	Collect Cape ivy and native *Senecio* test plants at sites in south-eastern Oregon with Veva Stansell
June 5	Meadowview	represent ARS at CalEPPC board meeting
June 6	Pt. Reyes	represent EIW at Marin Weed Management meeting
June 15	Monterey	collect *Senecio* test plants at Ft. Ord with Chuck Haugen
June 16	Carmel	Present invited talk "Biological Control of Cape Ivy" to the annual meeting of the Rio Padres Reserve Association. As a result, the Association votes to contribute $1500 to the Cape ivy Biocontrol Project.
July 4-5	Cave Junction	collect *Senecio* test plants at Fiddler Mtn. with Andrea Williams

	Oregon	
July 9	Monterey	collect *Senecio* test plants at Ft. Ord with Chuck Haugen
July 10	Carmel	Collect Cape ivy samples for pre-release surveys of herbivores at proposed release sites in Rio Pedros Reserve and Carmel Highlands
July 15-19	Minneapolis	Attend annual meeting of Aquatic Plant Management Society; present talk "Progress towards control of Cape ivy, a serious riparian weed in western USA".
Jul. 21	Calistoga	Lead Weed/Native Hike #4 at Sugarloaf Ridge for the San Francisco Bay Sierra Club
Aug. 1-6	Bozeman MT	Presented invited talk on "Code of Best Practices for Classical Biological Control of Weeds" at the Practice of Biological Control Symposium.
Aug. 11	Pt. Reyes	Lead Weed/Native Hike #5 at Central Pt. Reyes for the San Francisco Bay Sierra Club
Aug. 20	Monterey	collect *Senecio* test plants at Ft. Ord with Chuck Haugen
Aug. 21	Carmel	Collect Cape ivy samples for pre-release surveys of herbivores at proposed release sites in Rio Pedros Reserve and Carmel Highlands
Aug 23	Albany	Attend Solano Stroll Planning Committee meeting
Aug 29	Pt. Reyes	represent EIW at Marin-Sonoma Weed Management Area meeting
Sep. 8	Pacifica	Lead Weed/Native Hike #6 at Montara Mtn. for the San Francisco Bay Sierra Club
Sep. 9	Albany	Organize, set-up, and man the EIW posters at Solano Stroll
Sep. 16-18	El Cerrito	Host visiting Turkish scientists, Nezihi & Sibel Uygur
Sep. 26	Albany	Attend Quarantine Committee meeting

Oct. 5-7	San Diego	Attend 10th Annual CalEPPC Symposium: present invited talk "Update on the Cape Ivy Bio-control Project"; serve as co-moderator for the "2-hr Cape ivy Breakout Session
Oct. 10-12	S. Lake Tahoe	Attend annual W-185 Meeting at Fallen Leaf Lake Lodge
Oct. 15-16	Carmel	Visit Cape ivy sites with Tracy Johnson, U.S. National Park Service biological control of weeds entomologist, Hawaii Volcanoes Park. Collect Cape ivy samples for pre-release surveys of herbivores at proposed release sites in Rio Pedros Reserve and Carmel Highlands
Oct 17	Albany	Host quarantine visit by Tracy Johnson
Oct. 20	Shelter Cove	Collect geographic data on this previously unknown Cape ivy infestation in southern Humboldt County
Oct. 22	Davis	Present invited seminar "Biological Control of Cape Ivy" to the UC-Davis Monday Morning Weeders Group
Oct 25	Albany	Host site visit by Jack Broadbent, CalTrans Contracts Specialist
Nov. 15	Davis	represent ARS at CalEPPC board meeting; resign from Board

2002 Meetings, Travel, & Training

Jan 15-16	Carmel	Collect Cape ivy samples for pre-release surveys of herbivores at proposed release sites in Rio Pedros Reserve and Carmel Highlands
Feb. 4	Berkeley	Present invited poster at Bay Area Creek Conference
Feb 13	San Pablo	Serve as judge at Contra Costa County Science Fair
Mar. 14	San Francisco	Serve as judge at the San Francisco Bay Regional Science Fair
Mar. 16	Stinson	Lead Weed/Native Hike #7 from Stinson Beach for the San Francisco Bay Sierra Club
Mar. 23	Berkeley	Complete "Wilderness First Aid" training

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Appendices

Appendix A. 2001 Insect shipments to USDA-WRRC quarantine laboratory.

Incoming file #	Shipment contained	Location collected	Date received	Notes (U = unsexed)
BCW-WRRC-2001-1001	103 *Digitivalva* pupae	Wilderness, South Africa	1-9-01	70 moths emerged and used to form colonies.
BCW-WRRC-2001-1002	35 *Parafreutreta regalis* galls	Cape Town, South Africa	1-9-01	23 flies (7 ♀, 9 ♂, 7 U) emerged, but environmental chamber malfunction causes many deaths. Surviving flies used to form colonies.
BCW-WRRC-2001-1004	15 *Parafreutreta regalis* galls	Cape Town, South Africa	4-25-01	55 flies emerged (29 ♀, 26 ♂) and used in tests and colonies.
EIWRU-2001-1015	92 *Parafreutreta regalis* galls	Cape Town, South Africa	8-22-01	467 flies emerged (253 ♀, 210 ♂, 4 U) and used in tests and colonies.
EIWRU-2001-1018	100 *Digitivalva* pupae	Wilderness, South Africa	11-6-01	75 moths emerged and used in tests and colonies.

Appendix B. *Parafreutreta regalis* no-choice/host added tests.

Test No.	Non-target test plants	Dates	Oviposited on	Notes
PA-1-1004	*Eury. pectinatus* *Sen. blochmaniae* *Sen. macounii* *Sen. triangularis*	4-26 to 5-3 2001	Cape ivy	3 females alive when CI added (4-30), 1 female alive at end of test. **CI had 3 galls**, 2 dissected 7-31: 1st had 1 pupal case (PC), 2nd had 1 dead pupa, 3rd had *Pa.* damage
PA-2-1004	*Sen. blochmaniae* *Sen. hybridus* *Sen. macounii* *Sen. triangularis*	4-27 to 5-4 2001	Cape ivy	3 females alive when CI added (5-1), 1 female alive at end of test. **CI had 2 galls**, 1 dissected 7-31: the gall had 2 PC
PA-3-1004	*Eury. pectinatus* *Sen. blochmaniae* *Sen. bolanderi* *Sen. triangularis*	4-30 to 5-7 2001	nothing	2-3 females alive when CI added (5-3), no females alive at end of test.
PA-4-1004	*Eury. pectinatus* *Sen. blochmaniae* *Sen. hybridus* *Sen. macounii*	4-30 to 5-7 2001	nothing	4 females alive when CI added (5-3), 2 females alive at end of test.

PA-5-1004	*Eury. pectinatus* *Sen. bolanderi* *Sen. breweri* *Sen. flaccidus*	5-8 to 5-15 2001	nothing	2 females alive when CI added (5-12), no females alive at end of test.
PA-6-1004	*Eury. pectinatus* *Eury. subcarnosum* *Sen. bolanderi* *Sen triangularis*	7-9 to 7-16 2001	nothing	2 females alive when CI added (7-12), no females alive at end of test.
PA-7-1015	*Adenocaulon bicolor* *Hedera helix* *Sen. bolanderi* *Sen. confusus*	8-22 to 8-29 2001	nothing	3 females alive when CI added (8-25), 1 or 2 females alive at end of test.
PA-8-1015	*Adenocaulon bicolor* *Erechtites glomerata* *Eury. subcarnosum* *Hedera helix*	8-22 to 8-29 2001	Cape ivy	4 females alive when CI added (8-25), 2 or 3 females alive at end of test. **CI had 4 galls**, 3 dissected 11-5: 1st had 1 PC, 2nd had 3 PC, 3rd had 2 PC and 2 dead adults
PA-9-1015	*Adenocaulon bicolor* *Erechtites glomerata* *Sen. bolanderi* *Sen. confusus*	8-22 to 8-28 2001	nothing	4 (?) females alive when CI added (8-25), all females escaped before end of test.
PA-10-1015	*Erechtites glomerata* *Eury. subcarnosum* *Sen. blochmaniae* *Sen. confusus*	8-22 to 8-27 2001	nothing	2 females alive when CI added (8-25), no females alive at end of test.
PA-11-1015	*Adenocaulon bicolor* *Erechtites glomerata* *Eury. subcarnosum* *Sen. confusus*	8-27 to 9-4 2001	Cape ivy	4 females alive when CI added (8-30), 2 females alive at end of test. **CI had 7 galls**, 5 split 11-5: 1st had 3 PC, 2nd had 1 dead adult, 3rd had 3 PC, 4th had 1 PC, 5th had *Pa.* damage, 6th split 11-29: it had 5 PC and 1 dead pupa
PA-12-1015	*Erechtites glomerata* *Eury. subcarnosum* *Sen. flaccidus* *Sen. triangularis*	8-28 to 9-4 2001	Cape ivy	4 females alive when CI added (8-31), 3 females alive at end of test. **CI had 7 galls**, 4 dissected 11-5: 1st had 8 PC, 2nd had 2 PC and 1 pupa, 3rd had 1 PC, 1 pupa and 1 dead adult, 4th had 4 PC
PA-13-1015	*Erechtites glomerata* *Hedera helix* *Sen. bolanderi* *Sen. flaccidus*	8-29 to 9-5 2001	nothing	4 females alive when CI added (9-1), 2 females alive at end of test.

PA-14-1015	*Adenocaulon bicolor* *Eury. subcarnosum* *Sen. flaccidus* *Sen. macounii*	8-29 to 9-5 2001	Cape ivy	4 females alive when CI added (9-1), 1 female alive at end of test. **CI had 6 galls**, 2 dissected 11-5: 1st had 3 PC, 2 pupae and 1 dead adult, 2nd had 5 PC, 1 pupa and 1 dead adult *Adenocaulon* had possible *Pa.* damage
PA-15-1015	*Adenocaulon bicolor* *Eury. subcarnosum* *Sen. flaccidus* *Sen. macounii*	9-5 to 9-12 2001	nothing	4 females alive when CI added (9-7), 3 females alive at end of test.
PA-16-1015	*Eury. subcarnosum* *Sen. confusus* *Sen. flaccidus* *Sen. macounii*	9-12 to 9-19 2001	Cape ivy	4 females alive when CI added (9-14), 3 females alive at end of test. **CI had 1 gall**, 1 gall dissected 11-29: it had 2 PC
PA-17-1015	*Adenocaulon bicolor* *Sen. blochmaniae* *Sen. confusus* *Sen. flaccidus*	9-17 to 9-24 2001	Cape ivy	4 females alive when CI added (9-20), 3 females alive at end of test. **CI had 4 galls**, 4 dissected 11-29: 1st had 2 PC, 2nd had 1 dead pupa, 2 live females and 1 live male, 3rd had 5 PC, 4th had 4 PC
PA-18-1015	*Eury. pectinatus* *Hedera helix* *Sen. blochmaniae* *Sen. confusus*	10-15 to 10-22 2001	Cape ivy	4 females alive when CI added (10-18), 2 females alive at end of test. **CI had 5 galls**
PA-1-3015	*Hedera canariensis* *Euryops pectinatus* *Sen. hybridus* *Sen. bolanderi*	1-10 to 1-17 2002	Cape ivy	4 females alive when CI added (1-13), 2 females alive at end of test. **CI had 3 galls**, 1 dissected 4-10, 2 dissected 4-24: 1st had 7 pupal cases, 2nd had 1 dead pupa, 3rd had 1 PC
PA-2-3015	*Hedera canariensis* *Euryops pectinatus* *Sen. hybridus* *Sen. bolanderi*	1-14 to 1-22 2002	nothing	4 females alive when CI added (1-17), 1 female alive at end of test.
PA-4-4015	*Petasites frigidus* *Sen. breweri* *Sen. blochmaniae* *Sen. ganderi*	3-4 to 3-11 2002	Cape ivy	3 females alive when CI added (3-7), 1 females alive at end of test. **CI had 3 galls**, MORE
PA-5-4015	*Sen. bolanderi* *Sen. ganderi* *Sen. ganderi* *Sen. hybridus*	3-7 to 3-14 2002	Cape ivy	4 females alive when CI added (3-11), 2 females alive at end of test. **CI had 10 galls**, MORE

PA-6-4015	*Sen. vulgaris* *Sen. jacobaea* *Sen. breweri* *Luina hypoleuca*	3-11 to 3-18 2002	Cape ivy	3 females alive when CI added (3-14), 3 females alive at end of test. **CI had 13 galls**, MORE
PA-8-4015	*Sen. breweri* *Sen. vulgaris* *Sen. jacobaea* *Luina hypoleuca*	3-18 to 3-25 2002	Cape ivy	3 females alive when CI added (3-21), 3 females alive at end of test. **CI had 11 galls**, MORE

Appendix C. *Digitivalva* new sp. host range tests
(Test types: C = choice, NC = no-choice, NCHA = no-choice/host added).

Test No.	Test type	Non-target test plants	Dates	Oviposited on	Notes
DI-1-2001	C	*Sen. bolanderi* *Sen. macounii*	4-23 to 5-2 2001	Cape ivy	1 DI alive at end of test.
DI-2-2001	C	*Sen. triangularis*	4-24 to 5-7-01	nothing	1 DI alive at end of test.
DI-3-3001	C	*Sen. bolanderi*	6-14 to 6-21-01	nothing	no DI alive at end of test.
DI-4-3001	NC	*Sen. triangularis*	6-15 to 6-22-01	nothing	4 DI alive when CI added (6-19) 2 DI alive at end of test.
DI-5-3001	NC	*Sen. flaccidus*	6-18 to 6-25-01	nothing	5 DI alive when CI added (6-22) 3 DI alive at end of test.
DI-6-3001	NC	*Sen. blochmaniae*	6-18 to 6-25-01	Cape ivy	4 DI alive when CI added (6-22) 4 DI alive at end of test.
DI-7-3001	NC	*Pet. frigidus*	6-18 to 6-25-01	nothing	4 DI alive when CI added (6-22) 2 DI alive at end of test.
DI-9-3001	NC	*Sen. macounii*	6-20 to 6-28-01	nothing	5 DI alive when CI added (6-25) 5 DI alive at end of test.
DI-10-1018	NCHA	*Eury. subcarnosum* *Hedera helix* *Sen. confusus* *Sen. triangularis*	11-13 to 11-26-01	Cape ivy	7 DI alive when CI added (11-16) no DI alive at end of test. 2♀ & 2♂ adults emerged from Cape ivy
DI-11-1018	NCHA	*Eury. pectinatus* *Eury. subcarnosum* *Sen. hybridus* *Sen. macounii*	11-13 to 11-26-01	Cape ivy	7 DI alive when CI added (11-16) 4 DI alive at end of test. 4♀ & 3♂ adults emerged from Cape ivy
DI-12-1018	NCHA	*Eury. pectinatus* *Hedera helix* *Sen. bolanderi* *Sen. confusus*	11-14 to 11-26-01	Cape ivy	7 DI alive when CI added (11-20) 2 DI alive at end of test. 2♀ adults emerged from Cape ivy

DI-1-2018	NCHA	*Sen. jacobaea* *Sen macounii* *Sen. triangularis* *Sen. hybridus*	1-14 to 1-23 2002	Cape ivy	8 DI alive when CI added (1-17) 1 DI alive at end of test. 4♀ + 5♂ adults emerged from Cape ivy
DI-2-2018	NCHA	*Sen. jacobaea* *Sen triangularis* *Sen macounii* *Sen brewerii*	1-28 to 1-31-02	nothing	5 DI alive when CI added (1-31) 3 DI alive at end of test.
DI-3-2018	NCHA	*Sen. confusus* *Sen. jacobaea* *Sen. hybridus* *Sen. breweri*	2-5 to 2-13-02	Cape ivy	8 DI alive when CI added (2-8) 5 DI alive at end of test. 4♀ + 6♂ adults emerged from Cape ivy
DI-4-2018	NCHA	*Eury. pectinatus* *Eury. subcarnosum* *Sen. macounii* *Sen. breweri*	3-18 to 3-28-02	Cape ivy	8 DI alive when CI added (3-21) 4 DI alive at end of test. 17♀ + 14♂ adults emerged from Cape ivy
DI-5-2018	NCHA	*Hedera canariensis* *Luina hypoleuca* *Sen. jacobaea* *Sen. triangularis*	3-22 to 4-1-02	nothing	8 DI alive when CI added (3-26) 1 DI alive at end of test.
DI-6-2018	NCHA	*Luina hypoleuca* *Sen. vulgaris* *Sen. bolanderi* *Sen. breweri*	3-25 to 4-3-02	nothing	8 DI alive when CI added (3-29) 2 DI alive at end of test.
DI-7-2018	NCHA	*Luina hypoleuca* *Sen. vulgaris* *Sen. bolanderi* *Sen ganderi*	3-28 to 4-5-02	Cape ivy	8 DI alive when CI added (4-1) 3 DI alive at end of test. 3♀ + 4♂ adults emerged from Cape ivy

Appendix D. 1998-2001 Cardueae tribe plant surveys for *Chaetorellia succinea* in Oregon and California from 1998 to 2001.

Species	Common name	Date	County	Plants	Seedheads	% *Ch. succinea* infestation
Centaurea cyanus L. - introduced	bachelor's button	6/8/99	San Luis Obispo			0
		6/15/99	Shasta			0
		7/19/99	Shasta	10	333	0
		8/15/01	Siskiyou		548	0
		8/15/01	Siskiyou		1513	0
		8/15/01	Shasta		1053	0
		9/25/01	Plumas		1183	0
		9/26/01	Lassen		1931	0
		9/26/01	Modoc		1857	0
Cnt. maculosa Lam. - introduced	spotted knapweed	7/19/99	Shasta	2	1000+	0
Cnt. melitensis L. - introduced	No common name	9/20/99	Amador	30	369	21

		7/9/01	Monterey	21	748	5
		8/5/98	Humboldt	10	375	48
		8/5/98	Siskiyou	10	316	12
		9/17/98	Napa	0	317	52
		11/2/98	Butte	12	319	46
Cnt. solstitialis L. - introduced	yellow starthistle	7/19/99	Shasta	10	128	16
		7/19/99	Tehama	11	706	44
		9/20/99	Amador	45	1646	39
		9/20/99	San Joaquin	10	1000+	70
		10/6/99	Contra Costa	10	300+	61
		11/13/01	San Diego	5	257	0
		7/20/98	Nevada		30	0
Cirsium andersonii (A. Gray) Petrack	No common name	8/20/98	Nevada		30	0
		8/20/98	Nevada		100	0
		8/23/00	El Dorado			0
Cir. arvense (L.) Scop. - introduced	Canada thistle	1998	Plumas			0
		7/20/99	Modoc	11	1394	0
		8/4/98	Humboldt			0
		7/7/00	Clackamas (OR)	1		0
		7/19/00	Linn (OR)	1		0
Cir. brevistylum Cronq.	Indian thistle	7/19/00	Linn (OR)	10		0
		8/19/00	Del Norte			0
		8/19/00	Humboldt			0
		7/9/01	Monterey	10	173	0
		7/20/98	Nevada			0
		8/20/98	Nevada			0
		7/1/99	Nevada		292	0
Cir. canovirens Rydb.	gray-green thistle	7/1/99	Plumas			0
		7/19/00	Lake (OR)			0
		8/23/00	Alpine			0
		8/23/00	Alpine			0
		6/15/99	Kern		25	0
Cir. crassicaule (Greene) Jepson	No common name	1998 or 2000	Monterey			0
		8/6/98	Siskiyou	-	319	0
		8/18/98	Lassen	-	138	0
		8/18/98	Lassen	4	40	0
		8/19/98	Modoc	-	293	0
Cir. cymosum (Greene) J. T. Howell	peregrine thistle	6/8/99	Siskiyou		239	0
		6/8/99	Siskiyou			0
		6/8/99	Siskiyou		288	0
		7/19/99	Lassen	3	13	0
		7/19/99	Modoc	6	67	0
		7/19/99	Modoc	14	601	0
		7/1/00	Lassen			0
		8/5/98	Humboldt			0
		8/5/98	Humboldt			0
		8/20/98	Nevada	6		0
		7/20/99	Modoc	11	526	0
		8/19/00	Humboldt			0
Cir. douglasii DC	swamp thistle	8/19/00	Humboldt			0
		8/19/00	Humboldt			0
		8/29/00	Trinity			0
		8/29/00	Trinity			0
		8/30/00	Trinity			0
Cir. edule Nutt.	No common name	7/15/98	Douglas (OR)	8		0
Cir. loncholepis Petrak	La Graciosa thistle	5/27/99	San Luis Obispo		105	0

		5/5/99	Kern		60	0
		5/5/99	Kern		12	0
		5/26/99	Santa Barbara			0
Cir. occidentale var. *californicum* (A. Gray) Keil & Turner	California thistle	5/26/99	Santa Barbara		16	0
		5/26/99	Santa Barbara		59	0
		5/26/99	Santa Barbara	8	78	0
		5/26/99	Santa Barbara			0
		8/4/98	Trinity	5		0
		8/7/98	Siskiyou	8	117	0
		7/16/98	Siskiyou			0
		7/16/98	Lassen			0
		8/18/98	Lassen	5	180	0
		8/18/98	Shasta	3	38	0
		8/19/98	Modoc		60	0
		9/2/98	Plumas	9		0
Cir. occidentale var. *candidissimum* (Greene) J. F. Macbr.	snowy thistle	9/2/98	Plumas			0
		9/3/98	Plumas	10		0
		7/19/99	Shasta	11	161	0
		7/20/99	Modoc	5	92	0
		7/22/99	Mono	12	291	0
		8/23/00	Alpine			0
		8/23/00	Alpine			0
		8/24/00	Mono			0
		8/29/00	Trinity			0
Cir. occidentale var. *occidentale* (Nutt.) Jepson	cobwebby thistle	6/15/99	San Luis Obispo			0
		7/9/01	Monterey	10	78	0
		8/5/98	Humboldt	-	392	0
		6/15/99	Kern		138	0
		6/15/99	Monterey			0
		6/15/99	Monterey	8	95	0
		6/15/99	Monterey		63	0
Cir. occidentale var. *venustum* (Greene) Jepson	venus thistle	6/15/99	Monterey		150	0
		6/15/99	San Benito	12	277	0
		7/9/01	Monterey	11	52	0
		??	Fresno			0
		??	Mendocino			0
Cir. occidentale hybrid	snowy thistle hybrid	8/23/00	Alpine			0
		8/19/98	Modoc	10	114	0
Cir. ochrocentrum A. Gray - introduced	yellowspine thistle	7/20/99	Modoc	18	121	0
		7/19/00	Lake (OR)	10		0
Cir. quercetorum (A. Gray) Jepson	brownie thistle	7/9/01	Monterey	13	45	0
		7/19/00	Linn (OR)	10		0
Cir. remotifolium (Hook.) DC.		7/19/00	Linn (OR)	10		0
		8/31/00	Curry (OR)	26	112	0
Cir. scariosum Nutt.	elk thistle	9/2/98	Plumas	4		0
		7/1/99	Plumas			0
Cir. undulatum (Nutt.) Spreng - introduced	wavyleaf thistle	7/14/98	Wheeler (OR)	6		0
		7/18/00	Wasco (OR)			0

		7/16/98	Siskiyou	10	298	0
		8/5/98	Humboldt	12	346	0
		8/18/98	Shasta	-	149	0
		7/20/99	Modoc	5	149	0
		1998 or 1999	Marin	20	20	0
		1998 or 1999	County?? (OR)		3000+	0
		1998 or 1999	San Luis Obispo	10		0
		1999	Marin	15	218	0
		1999	Marin	15	436	0
		1999	Marin	15	391	0
		1999	Marin	15	259	0
Cir. vulgare (Savi) Ten. - introduced	bull thistle	1999	San Luis Obispo	20	284	0
		2000	Marin	10	360	0
		2000	Marin	22	215	0
		2000	Marin	15	250	0
		2000	Marin	15	206	0
		2000	Marin	15	273	0
		2000	Marin	10	765	0
		2000	Marin	10	591	0
		2000	San Luis Obispo	10	398	0
		2000	San Luis Obispo	11	426	0
		2000	San Luis Obispo	10	410	0
		2000	Tulare	10	270	0
Cnicus benedictus L. - introduced	blessed thistle	7/9/01	Monterey	23	91	0

Appendix E. Cut leaf trial: plant species on which neonate *Diota rostrata* larvae have developed.

Species	Larval stage	Pupal stage	Total stage
S. angulatus	34	25	59
S. brachy	35	25	59
S. pleisto	35	26	62
S. tamoid	40	25	64
S. helmin	49	27	77
S. quin	56	26	82
S. oxy	53	25	79
Kleinia	63	25	88
Mikaniop	73	17	90
S. flacid	43	17	60
Blackjack	69	19	88

Cut leaf trial: key species (summer)

Species	Larval stage	Pupal stage	Total stage
Delairea	16	9	25
S. angul	21	9	30
S. oxyo	21	9	29
S. flaccid	18	9	28

Appendix F. *Diota rostrata* development time on various plants.

	Larval stage	Pupal stage	Total no. of days
Delairea odorata			
Mean	16.3125	8.75	25.0625
Standard Error	0.325560414	0.232737	0.1929756
Median	16	9	25
Standard Deviation	1.302241657	0.930949	0.77190241
Sample Variance	1.695833333	0.866667	0.59583333
Minimum	14	7	24
Maximum	18	11	26
Sum	261	140	401
Count	16	16	16
Confidence Level (95.0%)	0.693916024	0.496068	0.41131801
Senecio angulatus			
Mean	20.86666667	8.666667	29.5333333
Standard Error	0.215288658	0.186871	0.23637474
Median	21	9	29
Standard Deviation	0.833809388	0.723747	0.91547542
Sample Variance	0.695238095	0.52381	0.83809524
Range	3	2	3
Minimum	19	8	28
Maximum	22	10	31
Sum	313	130	443
Count	15	15	15
Confidence Level(95.0%)	0.461748659	0.400798	0.50697384
Senecio oxyodontus			
Mean	20.5	8.6875	29.1875
Standard Error	0.456435465	0.284587	0.29181544
Standard Deviation	1.825741858	1.138347	1.16726175
Sample Variance	3.333333333	1.295833	1.3625
Minimum	18	6	28
Maximum	25	10	31
Sum	328	139	467
Count	16	16	16
Confidence Level (95.0%)	0.972869762	0.606583	0.62199027
Senecio flaccidus			
Mean	18.4375	9.25	27.6875
Standard Error	0.240983229	0.295804	0.44458548
Standard Deviation	0.963932916	1.183216	1.77834192
Sample Variance	0.929166667	1.4	3.1625
Range	3	4	7
Minimum	18	7	25
Maximum	21	11	32
Sum	295	148	443
Count	16	16	16
Confidence Level (95.0%)	0.51364391	0.630492	0.9476121

Appendix G. Lattice design used in the *Diota rostrata* oviposition trial.

List of test plants

No	Species	No	Species
1	*Senecio angulatus*	7	*S. oxyodontus*
2	*S. tamoides*	8	*S. flaccidus*
3	*S. brachypodus*	9	*Mikaniopsis cissampelina*
4	*S. pleistocephalus*	10	*Cineraria lobata*
5	*S. quinquelobus*	11	*Bidens pilosa*
6	*S. helminthioides*	12	*Delairea odorata*

Randomized list of test plants

No	Species	No	Species
1	S. brachypocus	7	*Bidens pilosa*
2	*S. quinquelobus*	8	*Delairea odorata*
3	*S. pleistocephalus*	9	*Mikaniopsis cissampelina*
4	*Cineraria lobata*	10	*S. helminthioides*
5	*S. angulatus*	11	*S. tamoides*
6	*S. flaccidus*	12	*S. oxyodontus*

Original				***Randomized blocks***				***Randomized rows***			
Block	***Rep X***			***Block***	***Rep X***			***Block***	***Rep X***		
X1	***1***	***2***	***3***	***X4***	***10***	***11***	***12***	***X4***	***10***	***12***	***11***
X2	***4***	***5***	***6***	***X2***	***4***	***5***	***6***	***X2***	***5***	***6***	***4***
X3	***7***	***8***	***9***	***X3***	***7***	***8***	***9***	***X3***	***8***	***9***	***7***
X4	***10***	***11***	***12***	***X1***	***1***	***2***	***3***	***X1***	***2***	***3***	***1***
Block	***Rep Y***			***Block***	***Rep Y***			***Block***	***Rep Y***		
Y1	*4*	*7*	*10*	***Y3***	*2*	*5*	*12*	***Y3***	*5*	*2*	*12*
Y2	*1*	*8*	*11*	***Y2***	*1*	*8*	*11*	***Y2***	*8*	*1*	*11*
Y3	*2*	*5*	*12*	***Y4***	*3*	*6*	*9*	***Y4***	*9*	*6*	*3*
Y4	*3*	*6*	*9*	***Y1***	*4*	*7*	*10*	***Y1***	*4*	*7*	*10*
Block	***Rep Z***			***Block***	***Rep Z***			***Block***	***Rep Z***		
Z1	*6*	*8*	*12*	***Z3***	*3*	*4*	*11*	***Z3***	*4*	*11*	*3*
Z2	*2*	*9*	*10*	***Z4***	*1*	*5*	*7*	***Z4***	*7*	*5*	*1*
Z3	*3*	*4*	*11*	***Z1***	*6*	*8*	*12*	***Z1***	*6*	*8*	*12*
Z4	*1*	*5*	*7*	***Z2***	*2*	*9*	*10*	***Z2***	*9*	*2*	*10*

- shaded squares denote test layout.